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Gene Expression Alterations in Activated Human T-Cells Induced by Modeled Microgravity

Nancy E. Ward,[1] Neal R. Pellis,[2] Semyon A. Risin,[3] and Diana Risin[4]*

[1]Wyle Life Sciences, Houston, Texas
[2]NASA, Johnson Space Center, Houston, Texas
[3]Department of Pathology and Laboratory Medicine,
University of Texas-Houston Medical School, Houston, Texas
[4]Human Adaptation and Countermeasures Office, NASA, Johnson Space Center, Houston, Texas

Abstract Studies conducted in real Space and in ground-based microgravity analog systems (MAS) have demonstrated changes in numerous lymphocyte functions. In this investigation we explored whether the observed functional changes in lymphocytes in MAS are associated with changes in gene expression. NASA-developed Rotating Wall Vessel (RWV) bioreactor was utilized as a MAS. Activated T lymphocytes were obtained by adding 100 ng/ml of anti-CD3 and 100 U/ml of IL-2 in RPMI medium to blood donor mononuclear cells for 4 days. After that the cells were washed and additionally cultured for up to 2 weeks with media (RPMI, 10% FBS and 100 U/ml IL-2) replacement every 3–4 days. Flow cytometry analysis had proven that activated T lymphocytes were the only cells remaining in culture by that time. They were split into two portions, cultured for additional 24 h in either static or simulated microgravity conditions, and used for RNA extraction. The gene expression was assessed by Affymetrix GeneChip® Human U133A array allowing screening for expression of 18,400 genes. About 4–8% of tested genes responded to MG by more than a 1.5-fold change in expression; however, reproducible changes were observed only in 89 genes. Ten of these genes were upregulated and 79 were downregulated. These genes were categorized by associated pathways and viewed graphically through histogram analysis. Separate histograms of each pathway were then constructed representing individual gene expression fold changes. Possible functional consequences of the identified reproducible gene expression changes are discussed. J. Cell. Biochem. 99: 1187–1202, 2006.

Key words: immunity; human lymphocytes; gravitational biology; space physiology; gene expression

Space flight results in profound effects on various physiological systems [Graebe et al., 2004], including a decline in cellular immune function [Leach et al., 1990; Cogoli, 1993a,b; Wu et al., 1993]. The immunological impairment observed in astronauts may result both from indirect effects on immunity through microgravity-induced changes in other systems—psychoneuroendocrine changes, for example [Meehan et al., 1993]—as well as from the direct effects of microgravity on immune cells.

Direct effects of microgravity on lymphocyte functions are well documented mostly in ground-based studies utilizing MAS [Cogoli, 1993c; Cogoli et al., 1993; Cooper and Pellis, 1998; Cooper et al., 2001; Risin and Pellis, 2001; Sundaresan et al., 2002]. Simulated microgravity has been proven to inhibit lymphocyte locomotion [Pellis et al., 1997] and affect expression of cell surface molecules [Cooper and Pellis, 1998], suppress polyclonal and antigen-specific lymphocyte activation [Cogoli et al., 1993; Risin et al., 1995; Cooper and Pellis, 1998; Pellis et al., 1999], selectively change protein kinase C (PKC) isoforms expression [Pellis et al., 1999; Risin et al., 1999], and inhibit activation-induced programmed cell death [Risin and Pellis, 2001]. Some of these effects were also confirmed in cell culture experiments

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*Correspondence to: Diana Risin, MD, PhD, NASA Johnson Space Center, 2101 NASA Parkway—Mail code SK, Houston, TX 77058-3696. E-mail: diana.risin-1@nasa.gov

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in real space conditions during Spacelab, Biokosmos, and Shuttle Missions [Cogoli et al., 1984, 1988, 1993; Cogoli and Tschopp, 1985; Pellis et al., 1997].

In this study we investigated whether the lymphocytes responses to microgravity are associated with changes in gene expression that would allow identification of major microgravity-sensitive genes.

As in our previous studies to simulate microgravity conditions, the NASA Rotating Wall Vessel (RWV) bioreactor was used. To identify the gene expression changes, we applied DNA chip technology allowing simultaneous screening for expression of numerous genes (18,400 genes with the array used). This methodology is based on a differential gene expression approach and high-density oligonucleotide arrays [Harrington et al., 2000; van Hal et al., 2000].

The experiments were performed in triplicate with T-cells obtained from different blood donors to minimize the possible input of biological variation in gene expression and discriminate the reproducible changes that are likely associated with the exposure to microgravity. Indeed, we found that exposure to modeled microgravity resulted in changes in expression of numerous genes. About 4–8% of tested genes responded by more than a 1.5-fold change in expression. Despite a considerable variability in gene response, a definite reproducible pattern was established. Thus, 89 genes responded by at least a 1.5-fold change in expression in all three experiments. Ten of these genes were upregulated and 79 were downregulated. A high number of these genes were found to be involved in fundamental cellular processes: signal transduction, DNA repair, apoptosis, and multiple metabolic pathways. A group of genes, directly related to immune and inflammatory responses, was also identified.

MATERIALS AND METHODS

Isolation and Activation of Human Lymphocytes

Buffy coats containing human peripheral blood mononuclear cells (PBMCs) were obtained from the Gulf Coast Regional Blood Center (Houston, TX). PBMCs were separated by centrifugation through a Ficoll-Hypaque gradient. The lymphocyte layer was extracted. The cells were washed with hanks balanced salt solution (HBSS) and resuspended in RPMI medium containing 10% fetal bovine serum (FBS).

Lymphocytes were activated by adding of 100 ng/ml of anti-CD3 (BD Biosciences, San Diego, CA) and 100 U/ml of IL-2 (Aldesleukin Proleukin—Chiron—Emeryville, CA) for 4 days. After that the cells were washed and placed in RPMI media containing 10% FBS and 100 U/ml IL-2 and cultured up to 2 weeks in static conditions with media replacement every 3–4 days. This procedure allowed to obtain pure population of activated T-cells. The purity of the cell population was confirmed by Flow cytometry analysis.

Simulated Microgravity and Static 1g Cell Cultures

To approximate MG conditions we used a specialized RWV culture system developed at the NASA-Johnson Space Center and commercially available from Synthecon, Inc. (Houston, TX). This very low-shear culture system randomizes gravitational vectors and approximates the microgravity environment by sustaining cells in continuous free fall [Schwarz et al., 1992; Tsao et al., 1992; Goodwin et al., 1993; Jessup et al., 1993; Unsworth and Lelkes, 1998]. This culture system has been successfully used in previous studies for the analysis of the effects of microgravity on PBMC locomotion on Earth and in parallel studies on Earth and in Space performed during space flight missions STS-54 and STS-56 [Pellis et al., 1997]. Preliminary activated T-cells (see above) were split in two portions. One was further maintained in static (1*g*) conditions and the other—placed in RWV bioreactor for 24 h at 37°C in atmosphere of 95% air and 5% CO_2 at 1×10^6 cells/ml cell density. RWV bioreactor was continuously turned at a speed of 22 rpm. Upon completion of the incubation the cells were washed with PBS and the cell pellets were frozen and placed in −80°C.

Isolation and Analysis of Total RNA

Total RNA was isolated utilizing the RNeasy isolation kit (Qiagen, Valencia, CA). Briefly, cell pellets were lysed and homogenized followed by the addition of ethanol to optimize binding of RNA to the spin column. Contaminants were removed via washing by centrifugation and total RNA was eluted in water. RNA was then

analyzed for quality utilizing the Agilent 2100 Bioanalyzer and the concentration was determined using the NanoDrop® ND-1000 Spectrophotometer.

Microarray Analysis

Microarray analysis was performed using the Affymetrix GeneChip® Human U133A array as described in the Affymetrix Analysis Technical Manual. The Affymetrix Hybridization Oven 640, the Affymetrix GeneChip® Fluidics Station, and the Affymetrix GeneChip® Scanner 3000 were utilized. The Human U133A array contains >22,000 probe sets corresponding to 18,400 genes. Microarrays were performed in triplicate using RNA isolated from the activated lymphocytes of three different blood donors. A cutoff was set at ≥1.5-fold for analysis and for identifying genes of interest with the criterion for significance <0.01. The results were submitted to the Gene Expression Omnibus (GEO) database along with sample and experimental design information. The series ID is GSE 4209, and the NCBI tracking system number is 15144051.

RESULTS

Three independent microarray experiments were performed utilizing activated T-cells isolated from different blood donors to identify gene expression changes occurring after 24 h exposure to simulated microgravity. About 4–8% of tested genes responded by more than a 1.5-fold change in expression. There was a sizeable variability in observed gene expression changes; however, 89 genes were identified demonstrating altered expression in all three experiments (Fig. 1A). Six of these genes (heat-shock 70 kDa protein 1A, diaphorase, guanylate binding protein 1, cold inducible RNA-binding protein, transferrin receptor, MHC class II HLA-DR-alpha) were assessed with more than one oligonucleotide probes that showed unidirectional statistically significant changes in expression. Of the 89 genes with altered expression 79 were downregulated and 10 were

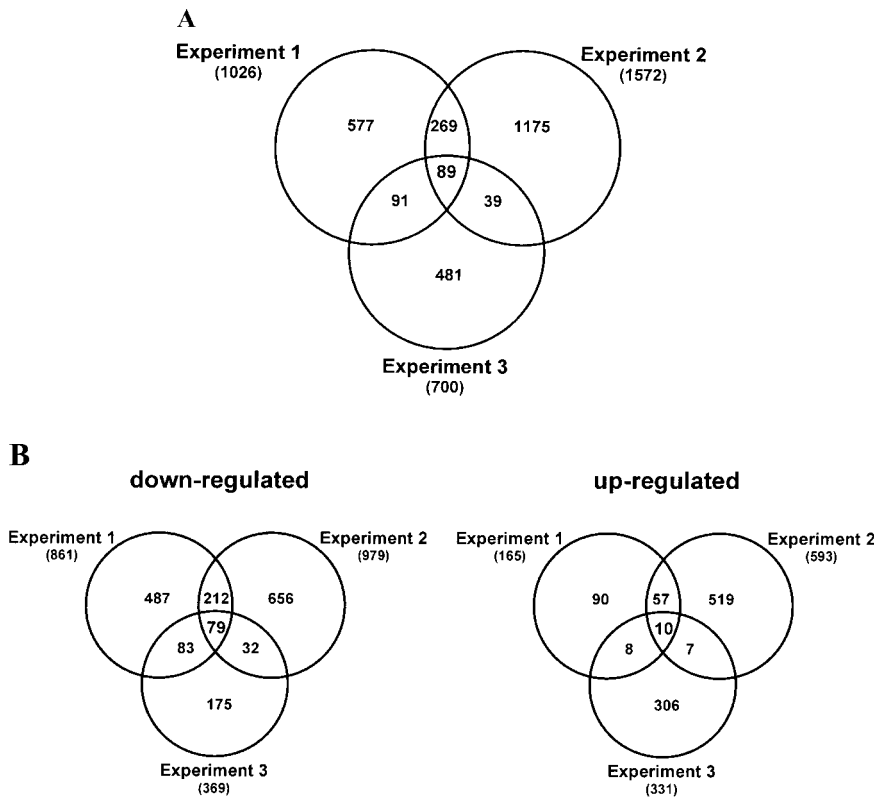

Fig. 1. Gene expression in activated human lymphocytes following 24 hours of simulated microgravity: (**A**) number of genes with altered expression in each of three separate experiments; (**B**) number of up- or down-regulated genes. *In the center* of each diagram is the number of genes up-regulated or down-regulated in all three experiments.

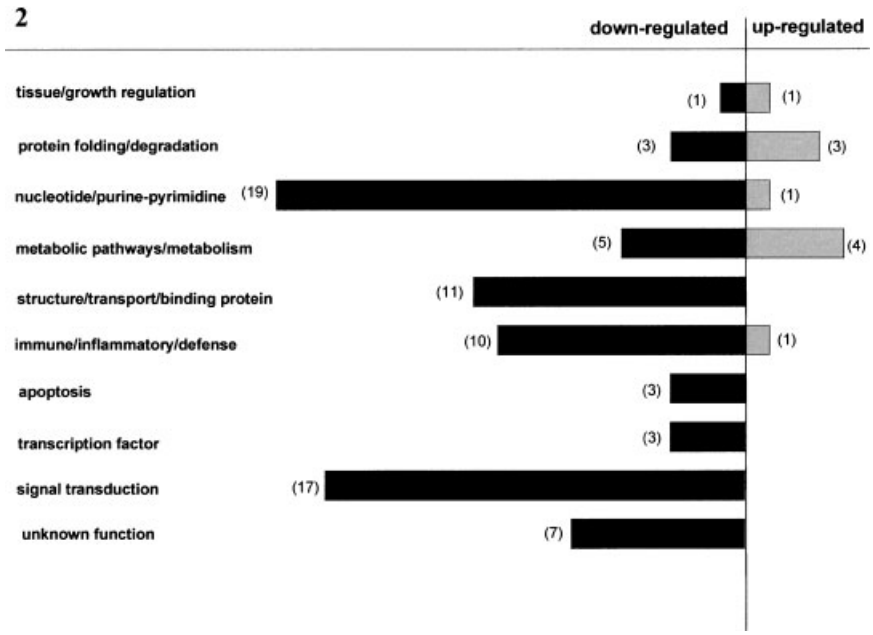

Fig. 2. Functional clustering of genes showing altered expression in response to 24 hours of simulated microgravity. Numbers in parentheses indicates the number of genes in that functional category up- or down-regulated.

upregulated (Fig. 1B). The genes demonstrating reproducibly altered expression were further categorized based on the available information into nine functional groups (Fig. 2). Separate histograms reflecting observed fold changes for individual genes were created within each functional group for a more detailed analysis (Fig. 3A–I).

Genes Related to Immune Response

In this functional group, 10 genes were downregulated and 1 gene was upregulated. The upregulated gene (IL7 receptor) is known to play a role in V(D)J recombination during lymphocyte development. It also controls the accessibility of the TCR by STAT5 and histone acetylation [Ye et al., 2001], and blocks apoptosis during differentiation and activation of T-cells [Maraskovsky et al., 1996, 1997]. The downregulated genes encode several important proteins. Granulysin is a cytolytic molecule demonstrating broad anti-microbial activity [Clayberger and Krensky, 2003]. Proteasome activator subunit 2 (PSME2) encodes the beta subunit of the 11S regulator of the immunoproteasome which is important in the processing of class I MHC peptides and induced by gamma interferon [Zhang et al., 1998; Sijts et al., 2002; Kloetzel, 2004]. The peroxiredoxin 4 gene encodes a cytosolic antioxidant enzyme shown to play a regulatory role in the activation of NF-KB [Kang et al., 1998; Fujii and Ikeda, 2002]. HLA-DRA functions in presenting peptides derived from extracellular proteins [Germain, 1994]. Lymphocyte antigen 75 is involved in the endocytosis of antigen by dendritic cells [Rajagopal et al., 2004]. The protein encoded by the IL18 receptor gene specifically binds IL18 and is essential for IL18-mediated signal transduction [Wu et al., 2003; Subramaniam et al., 2004]. Finally, DOCK2 gene product is a protein indispensable for lymphocyte chemotaxis [Reif and Cyster, 2002].

Genes Involved in Signal Transduction

In this group, all genes demonstrating altered expression were downregulated. The STAT1 and similar to STAT1 genes play a role in controlling cell cycle progression and also act as proapoptotic factors [Bromberg, 2002; Calo et al., 2003]. Regulator of G-protein signaling 1 (RSG1) modulates the activity of G-protein via acceleration of GTP hydrolysis and can also regulate the chemotaxis of lymphocytes to chemokines [Moratz et al., 2000, 2004; Zhong and Neubib, 2001]. Vaccinia-related kinase 1 is a novel Ser/Thr kinase that phosphorylates transcription factors related to stress responses

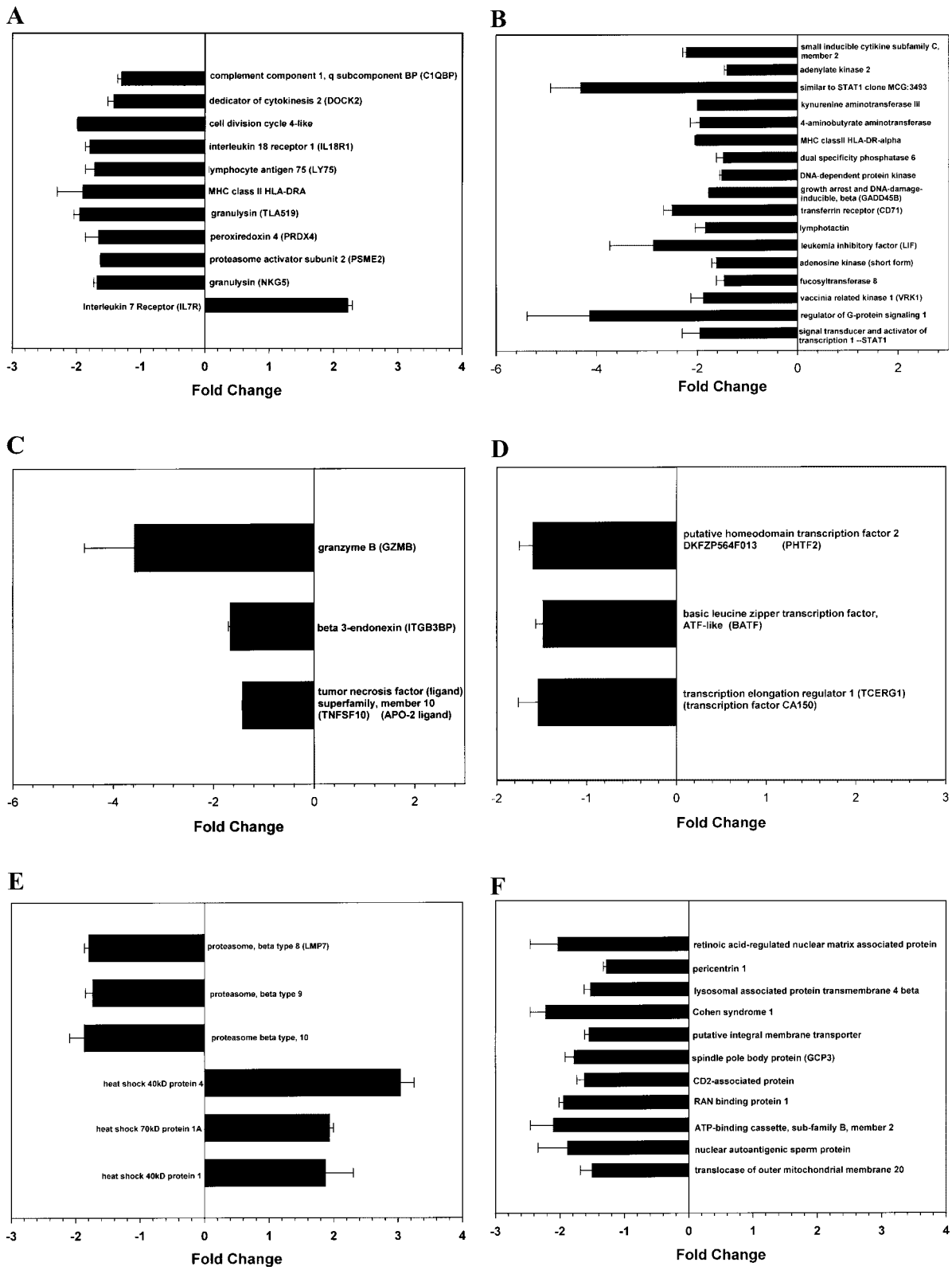

Fig. 3. Gene expression fold changes in response to 24 hours of simulated microgravity for different functional groups (means of three independent experiments ± SE): (**A**) genes related to immune response; (**B**) involved in signal transduction; (**C**) related to apoptosis; (**D**) transcription factor genes; (**E**) coding for proteins involved in protein folding and degradation; (**F**) structure-transport-binding protein genes; (**G**) tissue growth regulation genes; (**H**) involved in control of different metabolic pathways; and (**I**) genes for histones, nucleotide- and RNA-binding proteins, and DNA repair genes.

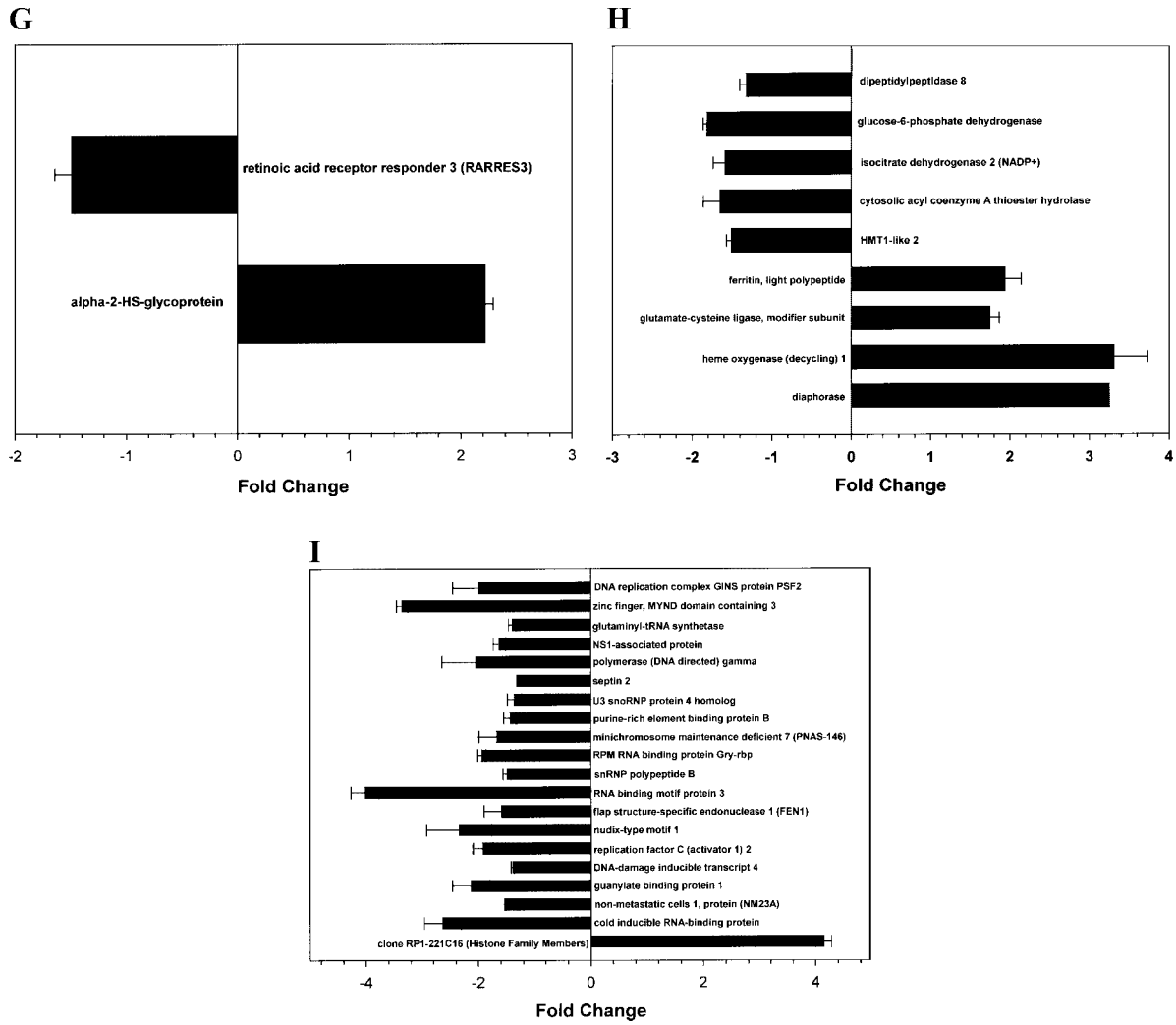

Fig. 3. (*Continued*)

such as p53, ATF, and c-Jun [Sevilla et al., 2004a,b; Vega et al., 2004]. The fucosyltransferase 8 (FUT8) gene product catalyzes the transfer of fucose from GDP-fucose to glycopeptides resulting in a range of glycoconjugates functioning in cell adhesion and lymphocyte circulation [de Vries et al., 2001]. Expression of the FUT8 gene may contribute to malignancy and metastatic capabilities of cancer cells [Ito et al., 2003] and also in liver disease [Miyoshi et al., 1999]. Adenosine kinase gene product is a phosphotransferase and serves as a regulator of both extracellular adenosine and intracellular adenine nucleotides [McNally et al., 1997]. The gene product of leukemia inhibitory factor (LIF) is a cytokine with a role in communication between neurons, activation of monocytes and macrophages, and inducing chemotaxis in immune cells [Auernhammer and Melmed, 2000; Metcalf, 2003; Chodorowska et al., 2004]. Lymphotactin (alpha and beta), is a C chemokine regulating cell trafficking through G protein-coupled receptors [Hedric and Zlotnik, 1998; Stievano et al., 2004]. Transferrin receptor binds the serum glycoprotein transferrin and is required for the iron delivery from transferrin to cells via an endocytotic process [Aisen, 2004; Taketani, 2005]. GADD45B mediates activation of the p38/JNK pathway in response to environmental stresses [Takekawa et al., 2002; Yoo et al., 2003] and is a feedback regulator perpetuating inflammatory signals [Lu et al., 2004]. DNA-dependent protein kinase is a nuclear Ser/Thr kinase activated upon DNA damage by both ionizing and UV irradiation as well as genotoxic stressors [Lee and Kim, 2002;

Yang et al., 2003, 2004]. Dual specificity phosphatase 6 negatively regulates members of the MAP kinase superfamily associated with cellular proliferation and differentiation [Camps et al., 2000]. ABAT, or 4-aminobutyrate aminotransferase, is responsible for the catabolism of gamma-aminobutyric acid (GABA) an important neurotransmitter. Deficiency in ABAT phenotype includes psychomotor retardation, hypotonia, hyperflaexia, lethargy, refractory seizures, and electroencephalogenic abnormalities [Medine-Kauwe et al., 1999]. The kynurenine aminotransferases are enzymes responsible for the synthesis of the endogenous neuroprotectant kynurenic acid [Rossi et al., 2004]. Adenylate kinase 2 is a mitochondrial enzyme involved in regulating adenine nucleotide composition within the cell and may also play a role in apoptosis [Bruns and Regina, 1977; Kohler et al., 1999; Van Loo et al., 2002].

Genes Related to Apoptosis

Only three genes with altered expression were identified in this group, all of which were downregulated. Granzyme B is crucial for induction of target cell apoptosis in immune response [Liu et al., 2003; Pardo et al., 2004; Shi et al., 2005]. Beta-3-endonexin induces apoptosis through a caspase-2-mediated pathway [Li et al., 2004]. Lastly is APO-2 ligand, which shows preferential apoptosis induction in transformed and tumor cells [Marsters et al., 1999; Kimberley and Screaton, 2004].

Transcription Factor Genes

In this group, three genes demonstrated a downregulation. BATF, a basic leucine zipper protein, belongs to the AP-1/ATF superfamily of transcription factors and is a regulator of AP-1/ATF transcription [Echlin et al., 2000; Deppmann et al., 2003]. The transcription factor CA150 gene encodes the protein transcription elongation regulator 1 which is a negative regulator of RNA polymerase II transcription elongation and also has a suggested role in HIV-1 gene regulation [Sune and Garcia-Blanco, 1999; Goldstrohm et al., 2001; Smith et al., 2004]. Putative homeodomain transcription factor 2 is a putative homeobox gene and a transcription factor predominately expressed in muscle [Manuel et al., 2000].

Genes Coding for Proteins Involved in Protein Folding and Degradation

This group showed three genes upregulated and three genes downregulated. All three genes upregulated were heat-shock proteins, HSP40, subfamily B, members 1 and 4, and HSP70 protein 1A. These proteins, in function with others involve them in protein folding, assembly and disassembly of protein complexes, and the stabilization of proteins against aggregation [Moseley, 2000]. The genes downregulated were all proteasome family members (subunits 8, 9, and 10), part of the 20S core unit and involved in cleaving peptides in an ATP/ubiquitin-dependent process. Expression of these genes is induced by gamma interferon, and the gene products replace subunits 5, 6, and 7, respectively, in the immunoproteasome [Coux et al., 1996; Nandi et al., 1997; Ciechanover, 1998; Murray et al., 1999].

Structure-Transport-Binding Protein Genes

All genes in this group were downregulated. Translocase of outer mitochondrial membrane 20, or TOM20 is part of a complex of mitochondrial proteins mediating the translocation of mitochondrial preproteins across the mitochondrial membrane [Yano et al., 2004]. The gene product nuclear autoantigenic sperm protein (NASP) is a histone transport protein [Richardson et al., 2000]. Transporter 1 (TAP) is a member of the superfamily of ATP binding cassette transporters involved in pumping degraded cytosolic peptides across the endoplasmic reticulum to be loaded onto MHC class I molecules [Koch et al., 2004]. RAN binding protein 1 gene encodes a protein which interacts specifically with GTP-charged RAN and acts as a negative regulator of Ran-GTP, or the RCC1 exchange factor for RAN which drives spindle assembly [Fiore et al., 2003]. The gene product CD2-associated protein is a multifunctional adaptor protein involved in the regulation of the actin cytoskeleton [Lehtonen et al., 2002]. Tubulin gamma complex-associated protein 3 is involved in the organization of the microtubule by the microtubule organizing centers (MTOCs) [Martin et al., 1997; Murphy et al., 1998]. Lysosomal-associated transmembrane protein 4 may play important roles in regulation of cell survival and proliferation and has been shown to be a potential protooncogene [Liu et al., 2004; Kasper et al., 2005].

Pericentrin1 is a component in the matrix of the centrosome and is involved in the establishment of microtubule arrays [Dictenberg et al., 1998; Perez-Ferreiro et al., 2004]. Retinoic acid-regulated nuclear matrix-associated protein (RAMP) is a serine/threonine-rich protein associated with the nuclear matrix protein. It translocates from the nucleus to the cytoplasm during mitosis and cytokinesis and may play a role in cellular proliferation [Cheung et al., 2001].

Genes Involved in Tissue Growth Regulation

Two genes changed expression levels in this group. Upregulated was Alpha-2-HS-glycoprotein, encoding a cytokine antagonist found in serum and bone and involved in regulation of chondrogenesis and osteogenesis in remodeling bone [Szweras et al., 2002]. Retinoic acid receptor responder 3 (RARRES3), which acts as a tumor suppressor or growth regulator [DiSepio et al., 1998; Jiang et al., 2005] was downregulated.

Genes Involved in Control of Different Metabolic Pathways

In this group, four genes were upregulated and five genes were downregulated. Upregulated genes were cytochrome b-5 reductase, heme oxygenase 1, glutamate-cysteine ligase, and ferritin light polypeptide. The cytochrome b-5 reductase gene encodes both the membrane bound and soluble forms of the enzyme which catalyze the oxidation of NADH or NADPH by various quinines and reduces methemoglobin to functional hemoglobin. Mutations in this gene have been shown to cause methemoglobinemias [Percy et al., 2002, 2005; Bando et al., 2004]. Heme oxygenase 1 is an enzyme essential in heme catabolism, cleaving heme to form biliverdin/bilirubin, CO and iron [Shibahara, 2003]. Glutamate-cysteine ligase, or gamma-glutamylcysteine synthetase is the rate-limiting step of glutathione synthesis, yielding the most predominant low molecular weight thiol in the cell, glutathione [Forman and Dickinson, 2003; Wu et al., 2004]. Ferritin light polypeptide is one of the two subunits making up ferritin, a major intracellular iron storage protein [Torti and Torti, 2002; Theil, 2003]. Downregulated genes were cytosolic acyl coenzyme A thioester hydrolase, isocitrate dehydrogenase 2, glucose-6-phosphate dehydrogenase (G6PD), HMT1 hnRNP methyltransferase-like 2, and dipeptidylpeptidase 8. Cytosolic acyl coenzyme A thioester hydrolase is also called BACH. The protein encoded by this gene hydrolyzes the CoA thioester of long-chain fatty acids [Yamada et al., 2002; Yamada, 2005]. Decreased expression of this gene has been associated with mesial temporal lobe epilepsy [Yang et al., 2004]. Isocitrate dehydrogenase 2 is a NADP(+) mitochondrial enzyme catalyzing the oxidative decarboxylation of isocitrate to 2-oxoglutarate and playing a role in intermediary metabolism and energy production [Yasutake et al., 2003]. The X-linked G6PD gene encodes a protein that is a key enzyme in the pentose phosphate pathway which functions to produce NADPH [Martini et al., 1986; Takizawa et al., 1986]. The HMT1 hnRNP methyltransferase-like 2 gene encodes a protein arginine methyltransferase (also called PMT1) which functions as a histone methyltransferase [Tang et al., 2000]. Dipeptidylpeptidase 8 cleaves peptide substrates at prolyl bonds and may play a role in T-cell activation and immune function [Abbott et al., 2000].

Histone, Nucleotide Binding, RNA-Binding Proteins, and DNA Repair Genes

One gene cluster on chromosome 6 was upregulated in this group. This cluster contains two genes for novel histone 4 family members, two genes for novel histone 1 family members, three genes for novel histone 2B family members, and a gene for a novel histone 2A family member L. The histones are responsible for nucleosome structure of the chromosomal fiber [Albig and Doenecke, 1997]. The remaining genes in this group were downregulated. Cold inducible RNA-binding protein can be induced by both mild cold stress and hypoxia and may play a role in cell response to various stressors [Nishiyama et al., 1997; Wellman et al., 2004]. Nucleoside-diphosphate kinase 1 was identified by reduced transcript levels in highly metastatic cells and exhibits both nucleoside diphosphate kinase activity and a metastatic suppressive activity [Gilles et al., 1991; Mac Donald et al., 1993; Hartsough et al., 2002]. Guanylate binding protein1 specifically binds guanine nucleotides and its expression is induced by interferon [Cheng et al., 1991; Prakash et al., 2000]. DNA-damage-inducible transcript 4, also known as RTP801, is stress

induced and may be involved in apoptosis and the regulation of cell growth via rapamycin [Shoshani et al., 2002; Corradetti et al., 2005]. The accessory protein replication factor C is necessary for the elongation of primed DNA templates by DNA polymerase delta and epsilon [Zhang et al., 1999]. Nudix-type motif 1 hydrolyzes oxidized purine nucleoside triphosphates, preventing their misincorporation into DNA and RNA during replication and transcription [McLennan et al., 2000; Mildvan et al., 2005]. Flap structure-specific endonuclease 1 (FEN1) removes overhanging flaps in DNA repair and processes 5′ ends of Okazaki fragments and is one of the essential proteins for cell-free DNA replication [Liu et al., 2004b; Kikuchi et al., 2005]. RNA-binding motif protein 3 gene is a member of the glycine-rich RNA-binding protein family and is induced by cold shock and hypoxia [Wellmann et al., 2004]. Small nuclear ribonucleoprotein polypeptide B encodes a nuclear protein found among small ribonucleoprotein particles which are involved in pre-mRNA splicing [Feeney et al., 1989; Gray et al., 1999]. Synaptotagmin-binding cytoplasmic RNA-interacting protein (SYNCRIP) is a cytoplasmic RNA-binding protein that interacts with ubiquitous synaptotagmin isoforms [Mizutani et al., 2000]. The protein encoded by the minichromosome maintenance protein 7 gene is highly conserved and essential for the initiation of genome replication as a component of the pre-replication complex and functions to initiate and elongate replication forks [Gladden and Diehl, 2003; Cortez et al., 2004]. The purine-rich element binding protein B gene product binds preferentially to the single strand of the purine-rich element PUR at the origins of replication and is implicated in the control of DNA replication and transcription [Gupta et al., 2003]. U3 snoRNP protein 4 is part of a complex required for the early cleavage steps in pre-rRNA processing [Granneman et al., 2003]. The septin 2 gene, also called septin 6, is a GTPase required for cytokinesis [Surka et al., 2002]. The polymerase (DNA directed) gamma gene encodes the catalytic subunit of mitochondrial DNA polymerase whose exonuclease and polymerase functions are essential for mtDNA maintenance [Spelbrink et al., 2000; Jazayeri et al., 2003]. Glutaminyl-tRNA synthetase forms a macromolecular protein complex and catalyzes the aminoacylation of tRNA by glutamine [Kim et al., 2000]. SET and MYND domain containing 3 is a histone methyltransferase that plays a role in transcriptional regulation as a member of an RNA polymerase complex [Hamamoto et al., 2004]. Lastly, DNA replication complex GINS protein PSF2 is a component of the novel replication complex GINS which is suggested to function for chromosomal DNA replication [Takayama et al., 2003; Obama et al., 2005].

DISCUSSION

Spaceflight conditions result in well-documented effects on several physiological systems including a decline in cellular immune function [Leach et al., 1990; Cogoli, 1993a,b; Wu et al., 1993]. MG is considered one of the major factors of space environment that affects numerous lymphocyte functions [Cogoli, 1993c; Cogoli et al., 1993; Pellis et al., 1997; Cooper and Pellis, 1998; Cooper et al., 2001; Risin and Pellis, 2001; Sundaresan et al., 2002]. To explore the possible involvement of genomic mechanisms in lymphocyte response to MG, microarray DNA chip analysis was utilized in this study in an attempt to identify gravity-sensitive genes. Upon the exposure of activated T-cells to modeled microgravity for a period of 24 h, 89 genes demonstrated reproducible more than 1.5-fold expression changes in comparison with control lymphocytes cultures at 1*g*. Ten of these genes were shown to be upregulated and 79 downregulated. The identified genes belong to different functional groups and their altered expression could be responsible for diverse functional changes. Some of these genes are of special interest considering the earlier established responses of lymphocytes to true and simulated MG conditions [Cogoli, 1993c; Cogoli et al., 1993; Pellis et al., 1997; Cooper and Pellis, 1998; Cooper et al., 2001; Risin and Pellis, 2001; Sundaresan et al., 2002]. Indeed, we identified a group of genes directly related to immune response (Fig. 3A). The only gene that was upregulated in this group is IL7R shown to play a critical role in the V(D)J recombination during lymphocyte development. It was suggested that defects in the protein encoded by this gene may be associated with the pathogenesis of the severe combined immunodeficiency [Roifman et al., 2000]. Knockout studies in mice showed that blocking apoptosis could be an essential function of this protein during differentiation and activation of T lymphocytes [Maraskovsky

et al., 1996, 1997]. The most functionally important among the downregulated genes (Fig. 3A) are: Lymphocyte antigen 75 (LY75), MHC class II HLA-DRA, Interleukin 18 receptor 1 (IL18R1), PSME2, and Granulysin (NKG5 and 519 transcripts). Lymphocyte antigen 75 (LY75) gene product is involved in endocytosis of antigen by dendritic cells [Rajagopal et al., 2004]. Downregulation of this gene could result in impaired antigen presentation and compromise the immune response. MHC class II HLA-DRA is expressed in antigen presenting cells and plays a central role in the immune system by presenting peptides derived from extracellular proteins [Germain, 1994]. The protein encoded by IL18R1 gene is a cytokine receptor that belongs to the interleukin 1 receptor family important in regulation of cellular immune response [Wu et al., 2003; Subramaniam et al., 2004]. PSME2 encodes the beta subunit of the 11S regulator of immunoproteosome that plays an essential role in processing of class I MHC peptides [Zhang et al., 1998; Sijts et al., 2002; Kloetzel, 2004]. Downregulation of this subunit could impair the antigen processing and affect the immune response. Granulysin is present in cytotoxic granules of cytotoxic T lymphocytes and natural killer cells. It is released from the granules upon antigen stimulation and executes anti-microbial activity [Clayberger and Krensky, 2003].

Some of the identified genes could be indirectly related to immune response by affecting fundamental cellular processes. Among those are genes involved in signal transduction and in control of cell proliferation and differentiation, in protein folding, transport and degradation, and in apoptosis. Among these genes we found particularly important for the immune response the genes encoding growth arrest and DNA-damage-inducible protein, beta (GADD45B), lymphotactin, LIF, and STAT 1. STAT 1 plays an important role in controlling cell cycle progression and acts as a proapoptotic factor and may be considered as a tumor suppressor [Bromberg, 2002; Calo et al., 2003]. In addition, mice deficient in STAT1 have been shown to be more sensitive towards viral and microbial infections [Durbin et al., 1996] and have enhanced oncogenesis [Kaplan et al., 1998]. We also observed a downregulation of RARRES 3 gene. The RARRES 3 is thought to act as a tumor suppressor [DiSepio et al., 1998; Jiang et al., 2005]. The downregulation of genes capable of acting as tumor suppressors would suggest the possibility of an increased susceptibility to oncogenesis in the microgravity environment that has to be explored. In the protein folding/degradation category, three genes were upregulated and three downregulated. Downregulated were three members of the proteasome B-type family (Proteasome beta type 8, 9, and 10). These three proteasome beta family member proteins make up part of the 20S proteasome core replacing certain catalytic subunits in the immunoproteasome [Coux et al., 1996; Nandi et al., 1997; Ciechanover, 1998; Murray et al., 1999]. Downregulation of these genes might affect antigen processing and antigen presentation. Upregulated genes in this group included heat-shock protein genes (40 kDa Protein 4, 70 kDa Protein 1A, and 40 kDa Protein 1). The proteins encoded by these genes function as molecular chaperones, involved in assembly and disassembly of protein complexes, in protein folding, renaturation of denatured proteins, and in prevention of protein aggregation and protein export [Moseley, 2000]. Upregulation of these genes could significantly affect the protein machinery with numerous consequences [Nollen and Morimoto, 2002].

Among apoptosis-related genes three genes were identified—Granzyme B, Beta 3-endonexin, and APO-2 ligand. All of these genes were downregulated. Two of the three genes—Beta 3-endonexin and APO-2 ligand—have direct relationship to apoptosis in lymphocytes. The Granzyme B is mainly involved in execution of the cytotoxic effects of CD8 lymphocytes by inducing apoptosis in the target cells [Liu et al., 2003; Pardo et al., 2004; Shi et al., 2005]. Beta 3-endonexin induces apoptosis through a caspase-2-mediated pathway that involves mitochondrial membrane permeabilization and does not require other caspases [Li et al., 2004]. APO-2 ligand gene encodes a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. The protein binds to several members of TNF receptor superfamily and triggers activation of MAPK8/JNK, caspase 8, and caspase 3. Apo-2 ligand (also called TRAIL) induces apoptosis primarily in transformed and tumor cells [Marsters et al., 1999; Kimberley and Screaton, 2004]. With the downregulation of three genes involved in the induction of apoptosis and upregulation of IL7 receptor gene involved in blocking of apoptosis, it is plausible that this combination could result in a

substantial inhibition of apoptosis in microgravity, as has been previously reported [Risin and Pellis, 2001].

We observed downregulation of genes that have the potential of affecting the integrity of the cellular cytoskeleton. The CD2-associated protein, which colocalizes with F-actin and functions as a scaffolding protein involved in regulating the actin cytoskeleton [Lehtonen et al., 2002] was downregulated, as was tubulin, or spindle pole body protein, involved in microtubule organization [Martin et al., 1997; Murphy et al., 1998] and pericentrin 1, a component of the filament matrix involved in the establishment of microtubule arrays [Dictenberg et al., 1998; Perez-Ferreiro et al., 2004].

Among the genes that respond to SMG there was also a significant group with unclear function in immune response and lymphocyte physiology. Nevertheless, they have well-established functions in other cellular systems and might predict space-exposure-related changes in these systems. Among those are two genes (Nudix and FEN 1) that could potentially affect DNA processing and repair. Nudix functions to hydrolyze oxidized purine nucleoside triphosphates, preventing their misincorporation into DNA and RNA [McLennan et al., 2000; Mildvan et al., 2005], and FEN 1 removes 5′ overhanging flaps in DNA repair [Liu et al., 2004b; Kikuchi et al., 2005]. The downregulation of these genes could result in an increase in damaged DNA in the microgravity of space. Interestingly, that we found also downregulated the G6PD gene. G6PD, as it is well known, by producing NADPH, the key electron donor in the defense against oxidizing agents, protects the cells from oxidative damage [Martini et al., 1986; Takizawa et al., 1986]. A deficiency in G6PD could result in diminished NADPH production, leading to an inhibition in the detoxification of oxidants such as hydrogen peroxide resulting in cellular damage by lipid peroxidation and protein and DNA oxidation.

In summary, lymphocytes exposure to simulated microgravity results in reproducible changes in the expression of multiple genes. These genes might be responsible for physiological changes encountered during space exploration. The obtained data provide a first insight into genomic mechanisms of physiological responses to MG and open a new avenue towards development of molecular countermeasures.

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