Proteomics and genomics of microgravity

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Nichols, Heather L., Ning Zhang, and Xuejun Wen. Proteomics and genomics of microgravity. Physiol Genomics 26: 163–171, 2006. First published May 16, 2006; doi:10.1152/physiolgenomics.00323.2005—Many serious adverse physiological changes occur during spaceflight. In the search for underlying mechanisms and possible new countermeasures, many experimental tools and methods have been developed to study microgravity caused physiological changes, ranging from in vitro bioreactor studies to spaceflight investigations. Recently, genomic and proteomic approaches have gained a lot of attention; after major scientific breakthroughs in the fields of genomics and proteomics, they are now widely accepted and used to understand biological processes. Understanding gene and/or protein expression is the key to unfolding the mechanisms behind microgravity-induced problems and, ultimately, finding effective countermeasures to spaceflight-induced alterations. Significant progress has been made in identifying the genes/proteins responsible for these changes. Although many of these genes and/or proteins were observed to be either upregulated or downregulated, however, no large-scale genomics and proteomics studies have been published so far. This review aims at summarizing the current status of microgravity-related genomics and proteomics studies and stimulating large-scale proteomics and genomics research activities.

MANY SERIOUS ADVERSE PHYSIOLOGICAL changes occur during spaceflight. Some of these include fluid redistribution, increased kidney filtration, sensory input changes, cardiovascular deconditioning (39), bone deterioration, muscle loss, and impaired immune system function (3, 7, 11, 63). Many of these pathophysiological adjustments cannot be counteracted adequately with physical exercise or nutritional supplementation, suggesting addition molecular mechanisms are responsible for the changes (47, 85). To develop highly effective countermeasures and prevent spaceflight-induced diseases, there is a critical need to understand the mechanisms of how microgravity causes these problems. Understanding the mechanisms of spaceflight-induced health problems may also help to provide insight into the pathophysiology of diseases occurring on Earth, such as osteoporosis, muscle atrophy, cardiovascular disease, and immune system dysfunction.

So far, the most prominent microgravity-induced cellular responses have been focused on bone (18, 50, 57, 72), muscle (37, 62, 118, 120), and immune system cells (28, 34, 46, 113). Animal and cell culture models have been studied in vivo or in vitro, in spaceflight or simulated microgravity conditions using tail-suspension models or land-based bioreactors. Many important metabolic and signaling pathways, in these and other cells, have been identified as being affected by microgravity, thereby altering cellular functions such as proliferation, differentiation, maturation, and cell survival. Recent studies have begun to focus on gene expression of cells cultured under microgravity. Messenger ribonucleic acid (mRNA) levels are studied to determine gene regulatory effects of cells as they attempt to acclimate themselves to the microgravity environment. However, the field lacks systematic investigation on microgravity-induced protein expression, which is the key information needed to ultimately unfold the mechanism behind microgravity-induced diseases. This review aims at summarizing the most recent advances in identifying gene and protein expression changes of several cell types, including bone, muscle, immune, and nervous system under microgravity conditions.

BONE CELLS

It is well known that biomechanical forces play critical roles in the development of the skeletal system (21, 29, 68, 83, 90, 95). Recently, it has been suggested that these forces may be as important as genetics in morphogenesis, tissue remodeling, and shaping of the tissue. Many studies have been performed to identify the alterations and mechanisms induced by skeletal unloading (microgravity) at the cellular level.

In normal bone there is equilibrium between bone formation and resorption. System hormones and local factors regulate bone remodeling, which involves cells, their proliferation, and progressive differentiation resulting in resorption of bone by osteoclasts and the deposition and mineralization of matrix by osteoblasts (68, 103). Microgravity results in the uncoupling of bone remodeling between formation and resorption that could account for bone loss (16). A decrease in osteoblast function is claimed to play a role in the process of spaceflight-induced bone loss. One mechanism in osteoblast differentiation is
governed by the regulation of runt-related transcription factor 2 (runx2), activator protein-1 (AP-1), and various other transcription factors. Upregulation of these factors leads to the upregulation of alkaline phosphates (ALP) and osteocalcin (OC) expression. If these are altered, bone loss can occur. More pathways may exist in controlling osteoblast differentiation and maturation.

When osteoblast histology was studied after spaceflight, an increase in less-differentiated (immature) and a decrease in more-differentiated (mature) osteoblasts were found, which suggests that microgravity blocks some of the differentiation pathways in osteoblasts (41). Researchers have also shown that osteoblasts and osteocytes are responsive to mechanical stimuli in vitro (29, 30, 88), and previous data have indicated that gene expression of growth factors and proteins (60, 84) are altered under microgravity conditions.

**Spaceflight studies.** Several effects have been observed in the gene/protein expression of bone cells cultured in spaceflight. These results have suggested a shift toward osteoblast dysfunction in microgravity. MC3T3-E1, mouse calvaria cells, flown on the STS-56 shuttle for 9 days showed a significant dysfunction in microgravity. MC3T3-E1 cells activated in microgravity used significantly less glucose than ground controls and had reduced prostaglandin E2 (PGE2) synthesis compared with controls. These data suggest that growth activation in microgravity results in reduced growth, causing reduced glucose utilization and reduced prostaglandin synthesis, significantly altering the actin cytoskeleton in osteoblasts (51). MC3T3-E1 cells have also been reported to have a depressed epidermal growth factor-induced c-fos expression under short-term microgravity conditions after time on the sounding rocket TR-1A6, whereas the phosphorylation of mitogen-activated protein kinase (MAPK) was not affected compared with ground-based controls. These results suggest that the action site of microgravity-induced effects in the signal transduction pathway may be downstream of MAPK (93).

No difference in morphology or DNA content was found in MG-63 osteocarcinoma cells flown for 9 days on the Foton 10 satellite. However, treatment with 1α,25-dihydroxyvitamin D3 [calcitriol, 1,25-(OH)2D3] and TGF-β2 resulted in a less increased ALP activity in cells under microgravity when compared with unit-gravity controls. Collagen-α1(1), ALP, and OC gene expression were also found to be lower in microgravity than controls using reverse transcription-polymerase chain reaction (RT-PCR) analysis (17). Message levels for growth factors TGF-β1 and latent TGF-β-binding proteins 1 and 2 have also been found to be altered in spaceflight (16). These results suggest that microgravity reduced differentiation of MG-63 cells under microgravity conditions in response to hormones and growth factors (17, 18).

Data have shown that spaceflight alters the mRNA level for several bone-specific proteins in rat bone. Changes in cell and nuclear morphology were observed as well as alterations in the expression of growth factors, including interleukin-6 (IL-6), insulin-like growth factor-binding proteins (IGF-BP), matrix proteins collagen type I, and OC, suggesting a decrease in osteoblast function (16). No change in DNA content was seen in bone-derived normal rat marrow stromal osteoblast cells cultured for 5 days on Spacelab flight STS-65, while PGE2 and IL-6 production increased. However, RT-PCR analysis determined an increase in prostaglandin endoperoxide H synthase-2 mRNA in microgravity cells, compared with control groups, suggesting that the arachidonic acid conversion pathway may be partially responsible for the increased PGE2 synthesis under microgravity (68).

PGE2 release in osteoblasts is associated with increased matrix synthesis (82, 119), and has also been found to increase the expression of IGFs in cells under mechanical strain (30). Insulin-like growth factor I (IGF-I) is a potent stimulator of bone formation that enhances cell proliferation and matrix formation in osteoblastic cells (15, 74). The effects of IGF-I on bone are determined by IGF-I concentration and its binding to IGF-BP (73). IGF-BP-3 has been shown to promote and also inhibit osteoblast proliferation (2, 32). Rats with skeletal unloading have shown impaired bone formation in the presence of insulin, indicating that the stimulatory effect of insulin on bone formation is lost by skeletal unloading (117). Treatment with 1,25-dihydroxyvitamin D3 of rat osteoblasts cultured for 4 or 5 days during a Space Shuttle mission showed an increase in mRNA levels for IGF-BP-3 and a decrease in IGF-BP-4 and -5. In addition, the glucocorticoid receptor mRNA levels in flight cultures increased compared with levels in ground controls (60). Altered IGF-BP production during spaceflight would modulate IGF action, further supporting the view that insulin impairment plays an important role in the deterioration of bone formation by microgravity (107). These cells also showed decreased TGF-β1 production, which resulted in a decrease in heat shock protein (HSP47), a collagen-specific molecular chaperone that controls collagen processing and quality. HSP70, which prevents stress-induced apoptosis, and HSP73, shown to prevent the pathological state induced by microtubule disruption, were also shown to be significantly reduced in microgravity cultures. These results suggest that microgravity differentially modulates the expression of molecular chaperones in osteoblasts involved in the induction and/or prevention of osteopenia in space (59).

**Ground-based studies (simulated microgravity).** Simulated microgravity is based on the hypothesis that sensing no weight would have similar effects to those of weightlessness (48). Several cell types have been studied using simulated microgravity bioreactors such as the three-dimensional clinostat and rotating wall vessel (RWV) systems. These systems utilize solid-phase rotation to maintain cells in suspension, where they experience randomized g-vectors (time-averaged) and low shear stress (45, 110). A suppression of osteoblast phenotype markers (87, 84) and modulation of apoptotic signals (76) have been observed in cells cultured in vector-averaged gravity conditions. Runx2 levels and AP-1 transactivation, key regulators of osteoblast differentiation and bone formation, were significantly reduced, along with mRNA levels for ALP, OC, core binding factor-α1, vitamin D receptor, receptor activator of NF-κB ligand (RANKL), and osteoprotegerin (OPG) (77, 79, 84). Human mesenchymal stem cells (hMSC) failed to display detectable levels for mRNA for major osteoblastic markers, including ALP, osteonectin, procollagen type I, and
runx2 after 7 days in simulated microgravity conditions, despite osteogenic induction (121). Collagen type I, the most abundant protein in the extracellular matrix of bone, was also dramatically reduced, while collagen-binding α2- and β1-integrin subunit expression was increased following 7 days in modeled microgravity (72). However, integrin signaling through focal adhesion protein (FAK), Ras, and extracellular signal-regulated kinase (ERK) was significantly reduced. Due to the potential role for FAK in osteoblast differentiation through the MAPK pathway (106), which signals ERK, essential for osteoblast differentiation (61), leading to runx2 activation, the reduction in integrin signaling likely contributes to reduced osteoblastogenesis (72). Suppression of RhoA in hMSC cultured in a rotary cell culture system has also been suggested to play a role in reduced osteoblastogenesis and enhanced adipogenesis in hMSC (71). Clinostat studies have also indicated that a reduction in osteoblast responsiveness to 1α,25-dihydroxyvitamin D3 might be involved in microgravity-induced osteopenia (77).

Simulated microgravity conditions induced a loss of ALP mRNA and activity in 2T3 preosteoblasts, indicating an inhibition of differentiation into osteoblasts (85). Microarray studies verified downregulation of runx2, which regulates OC expression (also decreased), osteomodulin, involved in bone matrix formation (12) and parathyroid hormone receptor 1, which promotes the release of Ca2+ from bone (109). An upregulation of osteolytic inducers, such as cathepsin K, was also seen. Cathepsin K is expressed mainly in osteoclasts, and its pathophysiological implications in osteoblasts is unclear (85), suggesting that it could be responsible for bone loss by directly stimulating osteoclasts and/or some undefined osteoblast-dependent mechanism.

Cultures of bone marrow stromal cells ST2 in vector-averaged gravity conditions revealed an increase in mRNA expression for RANKL and a decrease in levels for OPG, modulation of which may be one of the causes of osteopenia due to skeletal unloading (54). Researchers are now investigating OPG as a potential drug for treating osteoporosis and bone loss associated with metastatic bone cancer, as well as bone loss caused by extended exposure to microgravity (42).

MC3T3-E1 osteoblast-like cells cultured in alginate carriers in the National Aeronautics and Space Administration (NASA)-approved high aspect ratio vessel, had decreases in expression levels of OC, ALP, collagen type I, and runx2 and were more sensitive to apoptogens, such as staurosporine (11), when compared with controls. There was also a low level of antiapoptotic protein Bcl-2, as well as Akt protein, which regulates mitochondrial function, suggesting an inducement of apoptotic events in cells exposed to microgravity (11). Nakamura et al. (76) found similar effects of vector-averaged gravity on human osteoblastic cells. The ratios for mRNA levels of Bax (triggers release of cytochrome c from mitochondria)/Bcl-2 (blocks cytochrome c release) were increased significantly compared with 1G static controls. However, XIAP (antiapoptotic molecule) mRNA levels were also increased significantly, suggesting the modulation of both pro- and antiapoptotic molecules by simulated microgravity (weightlessness) (76).

Systematic investigation, including DNA microarrays and proteomics analysis, of the mechanisms involved in microgravity-induced bone loss is lacking. To this end, we have studied the effects of simulated microgravity on protein/gene expression of human osteoblast cells seeded on engineered scaffolds in vitro using an RWV bioreactor (80). Through the use of comparative proteomics combining liquid chromatography mass spectroscopy analysis with differential two-dimensional gel electrophoresis, various proteins have been found to be upregulated or downregulated under simulated microgravity conditions. Proteins involved in osteoblast differentiation and bone formation [osterix (Osx), prostatic acid phosphatase (PAP)] and cell-cell adhesion (catenin) were downregulated, whereas those implicated in apoptosis [TGF-β-inducible early growth response 2 (TIEG2)], stress (HSP70) and cell growth/remodeling [ornithine decarboxylase (ODC) antizyme] were upregulated. Calcineurin, activated by a sustained increase in intracellular Cu2+ levels (105), and albumin were also found to be upregulated in cells under simulated microgravity conditions (80).

Osx regulates the later stages of osteoblast differentiation and is required for bone formation. However, the molecular mechanisms underlying Osx expression are not completely understood. Cellular PAP has been shown to directly enhance differentiated (not proliferative) characteristics (synthesis of collagen and ALP) of bone cells in vitro (67). These data suggest that there is a dysfunction in the osteoblast differentiation pathway in microgravity. The downregulation of cell adhesion proteins, such as catenin observed in our study (80), may weaken junctions between cells, affecting cell-cell signaling as well as tissue integrity. TIEG2 overexpression has been shown to induce apoptosis in hamster epithelial cells (22), whereas HSP70 functions to prevent cell damage due to environmental stress. Upregulation of albumin, shown to inhibit MC3T3-E1 cell differentiation while stimulating proliferation (52), further suggests a blocked differentiation pathway in osteoblasts under simulated microgravity. However, its expression in nonhepatocytes is currently unknown (52). ODC anti-enzyme prevents accumulation of cellular polyamines, with differential expression found in osteoblasts and osteoclasts, upregulation in osteoclasts (96), suggesting its role in bone remodeling.

Although a few studies have been carried out on bone cells using genomics or proteomics approach, there is a lack of long-term investigation to date. Long-term studies up to 2–3 yr using DNA microarrays and proteomics approaches may provide inside information for developing effective countermeasures for long-duration missions, e.g., trips to Mars or long stays on the moon.

**MUSCLE CELLS**

Spaceflight has been shown to cause atrophy and reduction in force and power of skeletal muscle (13, 87). These changes are due primarily from a reduction in protein synthesis that is likely triggered by the removal of the gravitational load (36, 102). Contractile proteins are lost disproportionately to other cellular proteins, and actin thin filaments see a more significant decline than myosin thick filaments. A shift toward enhanced degradation of intracellular small nonmyofibrillar proteins and a degradation of extracellular structures has also been suggested through rat-specific microarray analysis (115). Steffen and Musacchia (100) noticed a decline in both myofibril and...
sarcoplasmic protein (total) in rats after 7 days in spaceflight. However, protein concentration remained unchanged compared with ground controls. Haddad et al. (44) observed similar results in rats after 9 days in spaceflight, although Baldwin et al. (6) found a significant decrease in myofibril protein concentration in slow-twitch muscle fibers, but not fast-twitch, after almost 13 days in spaceflight. Data suggest that spaceflight and hindlimb suspension (HS) cause a selective loss of contractile protein synthesis and slow-to-fast transitions in contractile and regulatory proteins (14, 33, 69, 114, 118). Decreased β-myosin heavy chain (MHC) protein and mRNA expression has been found in rats under varying time periods of spaceflight and HS (5, 37, 58, 69). Moreover, 9 days in spaceflight significantly increased all fast mRNA levels with only slight increases in slow mRNA levels (33). These results suggest that changes in transcription and translation are responsible for the reduction in protein synthesis. Yamakuchi et al. (118) found the expression of 42 genes changed in rats exposed to microgravity. Myocyte-specific enhancer binding factor 2C (MEF2C) and MEF2C-related genes were significantly decreased. However, after 9 days of ground recovery the expression of MEF2C increased in sites of regeneration, suggesting MEF2C could be a key transcriptional factor for skeletal muscle atrophy/regeneration under microgravity condition. Stein et al. (101) noticed a consistent decrease in gene expression of proteins involved in fatty acid oxidation and an increase in those involved in glycolic activity using Affymetrix GeneChip Expression analysis techniques and software. This supports previous data of slow-to-fast shift in MHC isoform expression since this expression is typically correlated with glycolic enzymatic activity.

RT-PCR and DNA microarray techniques have revealed differences in the expression of >50 mRNA levels in male rats after 17 days on the NASA-STS-90 Neurolab spaceflight (108). Twelve genes were upregulated, and 38 genes downregulated, including those for cell proliferation and growth factor cascades (p21, Cip1, retinoblastoma, MAPK3, MAD3, ras-related protein RAB2), indicating a downregulation of genes involved in muscle satellite cell proliferation (108). In a similar experiment Lalani et al. (62) noted an increase in myostatin mRNA and protein levels and a decrease in IGF-II mRNA levels, suggesting their contribution to muscle atrophy during spaceflight.

Nikawa et al. (81) used DNA microarray analysis techniques to examine 26,000 gastrocnemius muscle genes in space-flown rats. They found an imbalanced expression of mitochondrial genes with disturbed expression of cytoskeletal molecules, including putative mitochondria-anchoring proteins, A-kinase anchoring protein and cytoplasmic dynein, and an upregulated expression of ubiquitin genes, MuRF-1, Cbl-B, and Siah-1A. These expression patterns, along with mitochondrial dislocation and an increase oxidative stress-induced gene expression, suggest insufficient energy provision of the mitochondrial (81).

Researchers have also been investigating the effects of microgravity on cardiac muscle (1, 86). Decreases in contractile force and velocity, as well as, Ca^{2+}-dependent actomyosin ATPase activity were seen in cardiac cells of tail suspension rats (120). No change was seen in the expression of MHC, tropomyosin, troponin T, or troponin I isoforms. Interestingly, a fragment of cardiac troponin I showed increased amounts in the hearts of these rats, suggesting its role in cardiac muscle adaptation (120). Conner and Hood (21a) observed a significant increase in heart malate dehydrogenase (MDH) enzyme activity, accompanied by a 62% elevation in heart MDH mRNA levels after microgravity exposure. They also found that heart cytochrome c oxidase enzyme activity remained unchanged in rats exposed to microgravity. Their results, compared with skeletal muscle, demonstrate that the heart undergoes more significant mitochondrial adaptations in response to short-term microgravity conditions (86). A decrease in the metabolic activity of cardiac cells has also been seen (91). However, it has been demonstrated that cardiovascular function shows individual variability (4).

Overall, weightlessness has been shown to cause atrophy, reduced functional capacity, and increased fatigue in skeletal muscles, most likely due to a reduction in protein synthesis caused by changes in transcription and translation. Also, there seems to be a general trend toward slow-to-fast transitions in contractile and regulatory proteins. Future studies are needed to define the effects of long-term spaceflight on muscle function and possible countermeasures.

### IMMUNE SYSTEM CELLS

Immune system dysfunction due to exposure to microgravity has been documented as well (98, 99). Studies using ground-based models have demonstrated effects on immune system cells such as reduced activation/proliferation (113), altered cytokine production, and altered signal transduction (23). Spaceflight studies have also noted altered cytokine production (43), as well as altered distribution of peripheral immune cells (70). CDNA microarray analysis has identified 11 cytoskeletal genes that are regulated differently in space-flown versus ground control Jurkat cells, suggesting that the cytoskeleton may sense gravity at the single cell level (66). Simulated microgravity (microarray) studies of Jurkat cells revealed an inhibited induction of 91 genes compared with normal gravity controls (10), whereas 10 genes regulated by key signaling pathways were confirmed by RT-PCR to have altered induction. In addition, differences between flight and controls for genes encoding various proteins involved in metabolism, signal transduction, adhesion, transcription, apoptosis, and tumor suppression have also been identified (8, 46, 113).

IL-2 and IL-2 receptor interaction plays a critical role as a third, and final, signal in T-cell activation. In vitro cinostat analysis of mitogen-activated T lymphocytes revealed substantial decreases in IL-2 and IL-2 receptor gene expression in microgravity, thus inhibiting full T-cell activation (20, 113). Others (9, 10, 19, 23, 24, 34, 43, 89) have also noted marked suppression of IL-2 expression/secretion and responsiveness to phytohemagglutinin of T lymphocytes under microgravity conditions. However, this activation can be fully restored by direct activation of protein kinase C (PKC) with phorbol myristate acetate and ionomycin, agents that are able to bypass mechanisms at the cell surface and impart their effect inside the cell (PKC activation) (23, 113). PKC and protein kinase A (PKA) are early regulators of T-cell activation (10). Although some studies suggest that there may be a microgravity-induced PKC defect (19, 27, 94), the impairment in the T-cell activation process has been found to be upstream of PKC activation (23,
However, the translocation of individual PKC isoforms βII, δ, and ε is differentially altered under microgravity (40, 46). Moreover, the PKA signaling pathway, which regulates cAMP response element-binding and NF-κB transcription factors, has been found to be responsible, at least partially, for the loss of T-cell activation under microgravity (10).

Using differential proteomics techniques, Risso et al. (89) found decreases in IL-2 production and cell proliferation, possibly caused by an overexpression of cytoskeleton proteins, moesin and annexin 4, which may affect signal transduction pathways (112) in Jurkat cells under conditions similar to microgravity. Risso’s group also observed impaired CD69 function, reduced mitochondrial membrane potential, and a downregulation of ATP-dependent DNA helicase, nuclear RNA helicase, Ras GTPase-activating protein protein binding 1, 26S proteasome non-ATPase regulatory subunit-6, and proteasome activator complex subunit-3. The results further suggest the cells’ failure to enter the cell cycle, as well as reduced levels of nucleic acid production.

Organ- and cell-specific differences in gene/protein expression have also been reported (24, 34, 43, 70). Fluid shifts are the most probable cause of lymphocyte redistribution among organs, which influences the cells’ activation potential (43). Felix et al. (34) used ground-based antithorostatic suspension (AOS) to examine cytokine expression in the lymph nodes, serum, and spleen of mice (34). What they found was an increase in IL-1β and a decrease in IL-2 in all organs, an increase in tissue necrosis factor (TNF-α) in the lymph nodes, and an increase in IL-6 in the serum and lymph nodes of the AOS mice. Lymphocyte migration and homing is also affected by microgravity-induced fluid shifts (43). The percentage of cells expressing the integrins lymphocyte function-associated antigen (LFA)-1α and LFA-1β, adhesion molecules, increased in splenocytes of flight animals but decreased in lymph node lymphocytes. Increase in expression of TNF-α has also been seen in fetal thymus T cells cultured in simulated microgravity (116). Flow cytometry analysis of immune cells from astronauts used to positively identify the specific lymphocyte subsets exhibiting space flight-induced alterations in cytokine production showed a reduction in IL-2 production by CD3+ T cells, CD4+, and CD8+ T cells (24). These results suggest that the decrease in immune function is organ specific, rather than the result of a general systemic immune response.

More recently it has been argued that activation of T cells with concanavalin A, or other equivalent mitogens, with simultaneous exposure to microgravity may mask some of the effects of the microgravity alone on proliferating cells, due to the opposing stimuli (28). Degan et al. (28) used preactivated lymphocytes to examine any changes in cellular metabolism due to microgravity exposure. They found that microgravity slows metabolic activity. Lymphocytes were no longer able to counteract DNA damage induced by KBrO3 or bleomycin (78), and intracellular ATP concentrations were significantly reduced.

Human lymphocytes treated with 1.5 Gy of X-rays alone or in combination with the DNA synthesis inhibitor of 1-β-D-arabinofuranosylcytosine showed significantly higher increases of aberrant cells and, hence, total number of aberrations compared with parallel treatments performed on the ground (75). These studies provide some insight into the potentially cumulative effects of simultaneous exposure to microgravity and cosmic radiation during spaceflight (56).

Although significant research has been done on T lymphocytes, little is known about the effects of microgravity on B lymphocytes, their growth, or antibody production (104). However, transfected SP2/0 myeloma cell line P3A2 cells cultivated under microgravity in RWV bioreactors have been shown to synthesize a significantly greater amount of the human antibody (anti-TNF-α) than those in static and spinner flasks (38). In contrast, it has been reported that murine hybridoma cells cultivated under microgravity in near-Earth orbit experiments show negligible differences in monoclonal antibody production (IgG) compared with those cultivated on the ground (97). More extensive research is needed to determine the specific effects of microgravity on cell lines producing potential key therapeutic proteins.

Various up- or downregulated proteins have been found in T lymphocytes exposed to microgravity, effecting proliferation, activation, adhesion, cell death, signal transduction, and so on. Fluid shifts likely cause the organ- and cell-specific differences seen after spaceflight. Countering these fluid shifts will be an extremely challenging task. However, microgravity exposure of some cell lines has shown promising therapeutic potential.

NERVOUS SYSTEM CELLS

Neural and neuroendocrine gene/protein adaptations to microgravity have also been studied. Using matrix-assisted laser desorption ionization time-of-flight mass, Sarkar et al. (92) have shown the differential expression of metabolism and cytoskeleton proteins in the hippocampus of the mouse brains subjected to simulated microgravity. The presence of caspase-7 and DNA fragmentation has also been seen in astrocytes under simulated microgravity conditions (clinostat), suggesting programmed cell death of astrocytes upon exposure to microgravity (111). Day et al. (26) found that exposure to microgravity reduces glial fibrillary acidic protein expression in hippocampal astrocytes. Feuilloley et al. (35) noted a difference in the biosynthesis and/or release of atrial natriuretic factor-like peptides in discrete regions of the brains of frogs exposed to microgravity. Lelkes et al. (65) used biochemical, immunological, and molecular biological techniques, including cDNA microarrays, to analyze signal transduction, catecholamine contents, and gene expression in three-dimensional tissue-like constructs of PC12 pheochromocytoma cells grown for 20 days in RWVs. They found enhanced phenylethanolamine-N-methyl transferase expression, with subsequent catecholamine synthesizing enzyme activity. A downregulation of “neuronal” genes (GAP 43) and an upregulation of “neuroendocrine” genes (chromogranin A) was also observed, suggesting selective activation of signal transduction pathways leading to enhanced neuroendocrine differentiation of PC12 cells (64).

There is growing evidence suggesting that significant changes occur in the spinal cord in response to prolonged exposure to microgravity, with significant alterations in the processing of sensory information from the periphery (31). Cerebral cortex studies in tail suspended rats have found a significant decrease of synaptic vesicles in axons of neuromuscular junctions, degenerative changes, and also vacant axonal spaces (25). In addition, a reduction in the number of γ-ami-
nobotryic acid (GABA) immunoreactive cells was seen, suggesting a compensatory reaction due to changes in afferent information. GABA is an inhibitory neurotransmitter widely distributed in the neurons of the cortex, which contributes to motor control, vision, and many other cortical functions. Edgerton et al. (31) found changes in the neural mechanisms that control slow and fast extensor muscles, where “slow” muscles were less responsive during locomotion following spaceflight. A decrease in the oxidative enzyme activity of spinal motor neurons innervating slow-twitch fibers and of sensory neurons in the dorsal root ganglion has also been observed following exposure to microgravity (53). These results combined with the fact that mammals have been shown to have a decrease in plasma levels of bioassayable growth hormone, possibly released from the pituitary, during spaceflight and bed rest suggest a neuroendocrine control system sensitivity to prolonged unloading of weight-bearing muscle (31).

CONCLUSION

Understanding gene and/or protein expression is the key to unfolding the mechanisms behind, and ultimately, finding effective countermeasures to spaceflight-induced alterations. Significant progress has been made in identifying the genes/proteins responsible for these changes. Although many of these genes and/or proteins were observed to be either upregulated or downregulated, there is a lack of systemic study of gene and protein expression in individual cells exposed to microgravity. Models for long-term study of the effects of microgravity on cells in vitro and possible countermeasures are essential as we send astronauts on long-term missions, i.e., to Mars and back. In the future it will be important to design human bed rest and International Space Station studies to determine the optimal combination of countermeasures. In addition to modifications related to gravity alone, it will be important to understand the potential effects of coexposure to cosmic radiation (55) to provide the maximum possible protection to astronauts.

GRANTS

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