

A Scientific Role for Space Station Freedom: Research at the Cellular Level

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TERRY C. JOHNSON

Division of Biology, Kansas State University, Manhattan, KS 66506

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JOHN N. BRADY

Division of Cancer Etiology, National Cancer Institute, Bethesda, MD 20892

ABSTRACT

The scientific importance of Space Station Freedom is discussed in light of the valuable information that can be gained in cellular and developmental biology with regard to the microgravity environment on the cellular cytoskeleton, cellular responses to extracellular signal molecules, morphology, events associated with cell division, and cellular physiology. Examples of studies in basic cell biology, as well as their potential importance to concerns for future enabling strategies, are presented.

INTRODUCTION

We are at the threshold of a historic opportunity to explore the potential role of gravity and the biological responses, at a cellular level, to the microgravity environment. Since the activities and properties of all organs and tissues, of both plants and animals, are communal expressions of their cell components, cell biology lies at the basis of all life forms.

While gravitational forces can be experimentally increased and almost every other aspect of the life environment of plant and animal species controlled, the potential impact of Earth's gravity on living cells, tissues, and organ systems remains largely unknown.

There are at least four major reasons for studying gravitational biology at the cellular level: (1) to gain fundamental knowledge of the potential influences of the microgravity environment on the cellular functions of both plant and animal cells; (2) to relate the cellular activities, altered under gravity unloading conditions, to a better understanding of events on Earth — in unit gravity — that are associated with the regulation of cell proliferation, gene action, development, etc.; (3) to exploit altered functions that occur in microgravity to generate products that will improve the quality of life; and, (4) to provide accurate projections of those long-term influences of the microgravity environment on cellular functions that

may threaten future space exploration (Life Sciences Division Working Group, 1991).

Although gravitational cell biology is in its infancy, there are clearly numerous guideposts that indicate that the future holds many interesting surprises, both pleasant and unpleasant, regarding how both plant and animal cells will respond to gravitational unloading, whether space adaptation is possible at the cellular level, and what physiological processes in the intact species will be significantly altered as a result of cellular responses to reduced gravity.

The major barrier that is presently faced by gravitational biologists is the scarcity of flight opportunities available for scientific research. Of no less importance is the relatively brief duration that characterizes our opportunities for microgravity research. Brief parabolic episodes on aircraft offer valuable, but extremely limited, opportunities for biological research. In many cases, these flights offer little more than opportunities to test various flight hardware and to test concepts of experimental design. Orbiter flights have had durations of only a few days and minimal opportunities, with but a brief time available by busy crewmembers with their manifold responsibilities in flight to aid in experimentation, and frequent launch delays simply constrain many experimental designs with living cells.

Gravitational biology may mature as a science only when a dedicated science laboratory, like Space Station Freedom, is available for intensive and long-duration studies. A manned space station can be neither justified nor denied for reasons other than the scientific potential it promises. In fact, no scientific facility is necessary as an end in itself. It is the applications to future scientific advancement that drive the need for any new instrumentation in science and engineering. For instance, is there a need for the electron microscope to allow advancement of science? The answer is, of course, both yes and no. Scientific advancements can, and are, made in many areas of biological sciences without the use of the

electron microscope. However, if scientific progress in other areas requires a visual examination of cellular substructure, beyond the resolution of the light microscope, the electron microscope is an indispensable instrument. In a similar vein, there are several ways to study gravity unloading with cellular systems. However, there is no question that progress will continue to be slow, incomplete, and excruciating in the absence of a permanently orbiting scientific laboratory.

It is reasonable to expect the biological scientific community to provide justification for the significant international investment that will be required for the development and maintenance of Space Station Freedom. Perhaps the most convincing evidence can be found in the intriguing observations of cellular responses to gravity unloading that have already been made under less than satisfactory experimental opportunities.

It is already clear that microgravity has an impact on living systems, including normal gravitropic responses of plant root tips, the developmental program of amphibian species, the embryological development of certain avians, and the growth dynamics of unicellular microbes. We suspect, however, that the influence of unit gravity is much more pervasive than presently known, and that the absence of Earth's gravitational forces will have a magnitude of influence that may be a barrier to long-term survival of life forms, including human, in the hostile space environment.

In some cases cells may serve as gravity sensors, although in most cases cells are influenced primarily by the microgravity environment and the absence or reduction of buoyancy-driven microconvection currents. Many of these features have already been extensively reviewed (Halstead and Dutcher, 1987; Todd, 1989; Krikorian and Levine, 1991; Space Studies Board, 1991; Lewis and Hughes-Fulford, 1993), and the present report will focus on newer observations, concentrate on cellular activities that are more indirectly influenced by the microgravity environment, and provide a synthesis of the potential impact that this influence may have on the future of space life sciences.

GRAVITATIONAL BIOLOGY AT THE CELLULAR LEVEL

The overall objectives of gravitational biology at the cellular level encompass identification of cellular processes uniquely influenced by the full spectrum of gravitational forces, and the access by researchers to *g* forces equal to and less than unit gravity (Life Sciences Division Working Group, 1991).

The goals include measures to:

- (1) Identify how single cells sense gravity, including both direct and indirect (environmentally mediated) effects.
- (2) Identify how cells transduce gravitational stimuli and how they respond to both acute and long-term variations in gravitational force.
- (3) Develop model cell systems to describe processes and mechanisms by which cells respond to altered gravitational force.

THE EXTRACELLULAR MATRIX AND CYTOSKELETON AS A GRAVITATIONAL REACTIVE COMPLEX

Others have suggested that the presence or absence of gravitational forces may influence cell function by modifying structural components associated with the extracellular matrix, plasma membrane, and cytoskeleton (Ex-Me-Cy) (Todd, 1989; Cipriano, 1990; Spooner, 1992) (Figure 1). Table I lists some of the components of the Ex-Me-Cy. These cellular substructures represent large polymerized complexes and each is characterized by a considerable macromolecular instability and, therefore, continuous turnover of their macromolecular components. Their significant size, intermolecular interactions, and turnover rates provide properties that suggest that they are reasonable candidates for being influenced by gravitational forces (Todd, 1989). Furthermore, they are involved in a wide range of cellular and intercellular features, including: cell-cell communication; cellular attachment and aggregation; cellular morphology; signal transduction; cellular contractile properties and motility; endocytosis; exocytosis; ion fluxes; and, molecular interactions with the proteins, glycoproteins, and lipids that comprise the architecture of the fluid mosaic membrane.

The extracellular matrix and the cytoskeleton are integral complexes common to both plant and animal cells. Although the extracellular matrix and cytoskeleton are often considered independent entities, they are in fact intimately associated directly and indirectly through their organization with both surface and intracellular membranes. The extracellular matrix of plant cells is distinguished by celluloses, pectins, and lignins comprising the cell wall. The extracellular matrix of animal cells is distinguished by collagens, proteoglycans, and laminins. The cytoplasmic cytoskeleton of all cells is established by an array of interconnecting microtubules, intermediate filaments, and microfilaments. These structures are

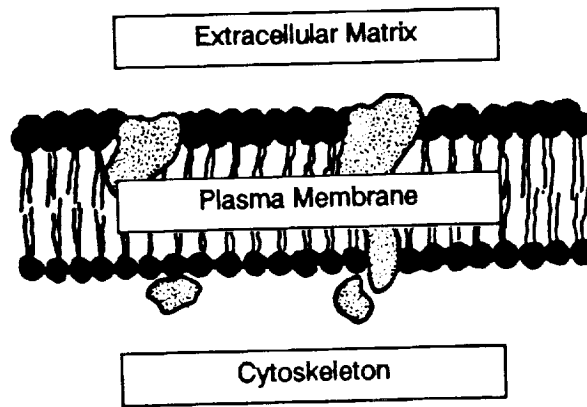


Figure 1. The Extracellular Matrix - Plasma Membrane - Cytoskeleton Complex.

Table I. Examples of the molecular components of the Ex-Me-Cy.

<u>Extracellular Material</u>	<u>Plasma Membrane</u>	<u>Cytoskeleton</u>
Collagens	Proteins	Microtubules
Proteoglycans	Glycoproteins	Microfilaments
Laminin	Lipids	Intermediate filaments
Fibronectin	Phospholipids	
Celluloses		
Pectins		
Lignins		

separated by a plasma membrane that provides a wide arrangement of binding sites for interactions with both the extracellular matrix and cytoskeletal complexes. In reality, therefore, these large polymerized substructures act in concert, as an integrated lattice, to regulate and influence a remarkable range of cellular activities. Many of these cellular activities are fundamental to the survival and function of the plant or animal, particularly in plant and animal tissues where the differentiated cellular components share a common overall purpose, yet bear distinctly separate responsibilities.

Many, if not most, of the cellular perturbations associated with the microgravity environment, therefore, may be consequences that will ultimately be associated with altered extracellular Ex-Me-Cy interactions. A brief overview of critical consequences associated with a prolonged exposure of cells to the microgravity environment includes events affiliated with cell-cell interactions and signal transduction, cell division, and immune responses.

Although most likely an indirect physiological consequence to gravity unloading, a microgravity response that involved the elements of the Ex-Me-Cy ultrastructure has been reported in animals subjected to a microgravity environment. Experimental observations from cellular elements of animal tissues from the Spacelab 3 mission in 1985 described cellular changes in tubulin and cytoskeleton synthesis and distribution, and changes in collagen secretion (Space Studies Board, 1991). Ultrastructural data from rats exposed to microgravity for 12.5 days on the Cosmos 1887 flight illustrated that perturbations in protofibrils (actin and myosin filaments) of rat cardiac tissue could result from gravity unloading (Philpott et al., 1990). Related observations were made on marked reduced myofibril yields from vastus intermedius muscles of rats from Cosmos 1887 (Baldwin et al., 1990). Interestingly, cytoskeletal elements associated with neuromuscular junctions have been shown to be altered when neurons and myocytes were cultured in a vector-free gravity environment (Gruener, 1991).

KC-135 and Consort I sounding rocket flights have been used to determine if a reduced gravity environment can influence assemblies of macromolecules that are associated with the cellular Ex-Me-Cy complex. Reduced gravity did alter the cell-free assembly of tubulin, collagen, and fibrin clot formation although the degree and direction of the influence was different for each of the molecules examined (Moos et al., 1990). Although these cell-free results may have been primarily a reflection of flow dynamics in the reduced gravity environment, and may not have been directly applicable to the intracellular cytoskeleton, flow dynamics in reduced gravity may play a key role in the maintenance and turnover of the extracellular matrix. In turn, these extracellular matrix changes could alter the molecular relationship of the cytoskeleton to the plasma membrane. It is also conceivable that the subsequent changes in the plasma membrane could transduce alterations in the cytoskeleton as it is associated with the cell surface.

These and other observations regarding the potential influence of a reduced gravity environment on the Ex-Me-Cy complex harbor potential consequences for an unusual number of cellular activities that are essential for maintaining biological integrity. In addition to the interesting basic science questions concerning gravitational impacts on living biological species, one cannot help but harbor concern that serious alterations in these functions may compromise the long-term survival of many biological species in the microgravity environment.

EXAMPLES OF CELLULAR ACTIVITIES THAT COULD BE COMPROMISED BY GRAVITATIONAL INFLUENCES ON THE EX-ME-CY COMPLEX

The cell surface plays a key role in cellular responses to extracellular cues such as peptide hormones, growth factors, growth inhibitors, and a myriad of signal molecules that provide critical information to this sensing organelle. In addition, direct cell-cell communication is mediated by macromolecules on the cell surface, and the Ex-Me-Cy complex responds to these signals in a manner that mediates cellular decisions that are essential to survival.

Cell-Cell Interactions

Cell-cell interactions play key roles in cellular communication that range from those that involve direct cell-cell contact to those that involve soluble ligands. Direct cell-cell contact plays an essential

role in the formation of cellular aggregation assemblies that are essential for normal tissue and organ development as well as for certain aspects of the normal immune response. Direct cell-cell contact also seems to be an important mechanism in inhibiting cell proliferation and the ability of tissues to maintain a steady-state turnover of cellular components without dangers of hyperplasia. Direct interactions are also essential to plant-microbe interactions that initiate the process that leads to symbiotic nitrogen fixation, and initial studies of this key interaction have been carried out in reduced gravity (DeBell et al., 1990; Urban, 1991).

Soluble ligands are the main communication links between cells over a distance, and these molecular cues provide a wide range of cellular responses, including alterations in cellular metabolism, stimulation of cell division, promotion or discouragement of neoplastic growth, cellular mobilization associated with inflammation, normal wound healing, and many key responses essential to the immune response.

Many features of cell-cell communication involve specific receptors associated with the plasma membrane. However, many of the responses described above also involve alterations in the entire Ex-Me-Cy complex, and are not limited to a simple molecular interaction of a ligand and receptor. For instance, the receptor-ligand complex can initiate a signal transduction cascade that involves the entire Ex-Me-Cy complex. The signal molecules can be components of the extracellular matrix that can interact with linking elements called integrins (Spooner, 1992). The subsequent cellular response may include the synthesis and secretion of extracellular matrix molecules that necessitates the participation of both the cytoskeleton and the plasma membrane.

The fluid mosaic plasma membrane is a dynamic structure that involves movement of its lipid and many of its macromolecular components at a remarkable rate. As reviewed by Edidin (1987), both lipid and protein components have a rotational diffusion that can be measured experimentally, and even single unaggregated 30 to 100 kDa proteins rotate on a time scale of microseconds. Addition of cytoskeletal proteins can increase the rotational correlation time as much as two-fold. Lateral diffusion constants for a wide variety of vertebrate membrane proteins have been measured, and generally are in the range of $D = 5 \times 10^{-9} \text{ cm}^2 \text{ sec}^{-1}$. These values are generally what would be predicted from the viscosity of synthetic phospholipid bilayers or estimated with the rotational diffusion of membrane proteins. However, many measurements of lateral diffusion made with native membrane preparations have been an order of magnitude less than what would be

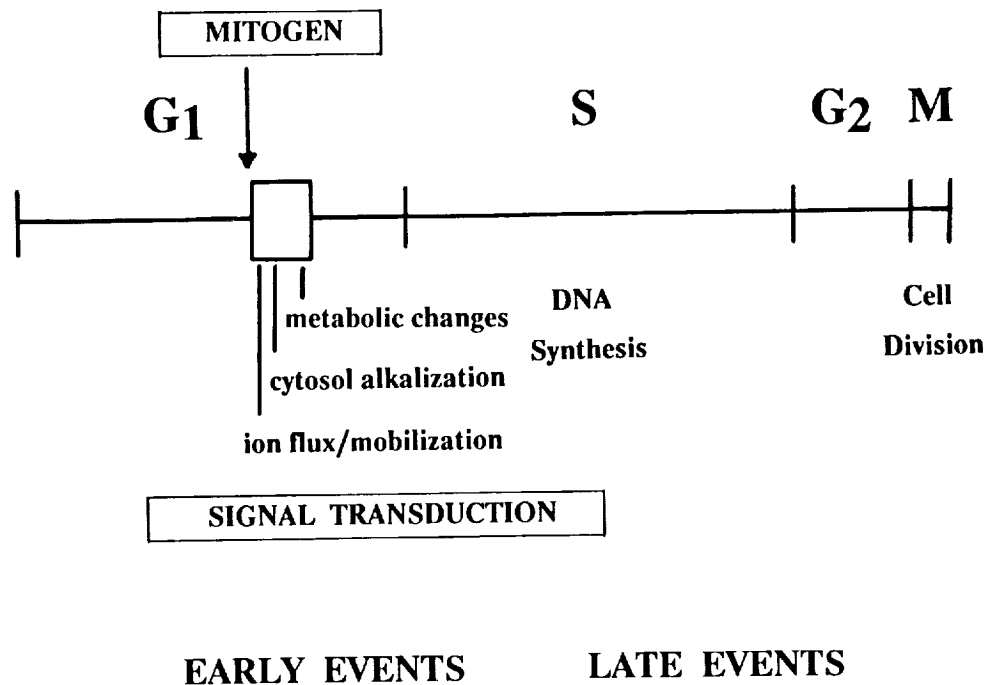


Figure 2. A linear display of the cell cycle and the most common arrest site at G₀/G₁.

predicted, and it is thought that interactions of the plasma membrane with cytoskeleton elements result in this discrepancy. Consistent with this possibility have been measurements of the lateral diffusion dynamics of membrane proteins in native membranes, in the absence of cytoskeletal proteins, that turn out to be 10 to 1000 times faster than that measured in native membranes in the presence of macromolecules of the cytoskeleton (Edidin, 1987). Even molecules diffusing as slowly as $10^{-13} \text{ cm}^2 \text{ sec}^{-1}$ can transverse the cell within a few hours. Of course, all membrane proteins and glycoproteins do not have the same freedom of motion. Many are anchored or clustered by elements of the extracellular matrix and cytoskeleton, and remain relatively quiescent compared to those macromolecules free to diffuse in the inner and outer sides of the lipid bilayer.

Cell Division

There is little question that the decision of a eukaryotic cell to divide or not to divide is a summation of external signals that involves the action of both growth factors and growth inhibitors. Both promoters and inhibitors of cell division influence cellular metabolism by binding to specific cell surface receptors that are most likely residents of the plasma membrane. Growth inhibitory molecules maintain the cells ar-

rested in the G₀/G₁ phase of the cell cycle (Figure 2) and growth factors, when overcoming these inhibitory influences, drive the cells into the S phase where DNA and histones are synthesized in preparation for cell division (Pardee, 1989).

The interaction between inhibitory and stimulatory ligands is extremely complex and remains the subject of intensive ground-based research. There are several pathways involved in signal transduction, as related to the control of cell proliferation, and this will be a key focus for future studies in the reduced gravity environment. In general, the binding of growth factors to the cell surface initiates a metabolic cascade that includes ion fluxes, release of Ca^{2+} from internal membrane stores, an alkalization of cytosol, metabolism of polyphosphate inositol, and the phosphorylation of cytosolic and nuclear proteins (Figure 3). The cascade includes the induction of specific gene expression that provides a state of competence for subsequent reentry of the cell to the cell cycle (Johnson and Sharifi, 1989; Toole-Simms et al., 1991; Fattaey et al., 1991; Edson et al., 1991).

As previously reviewed (Lewis and Hughes-Fulford, 1991), cell division of both plants and animals does appear to be significantly influenced in the microgravity environment. Several experiments have shown that prokaryotic bacteria and single plant cells appear to proliferate more rapidly in space than ground-level controls. Although there may be a multitude of

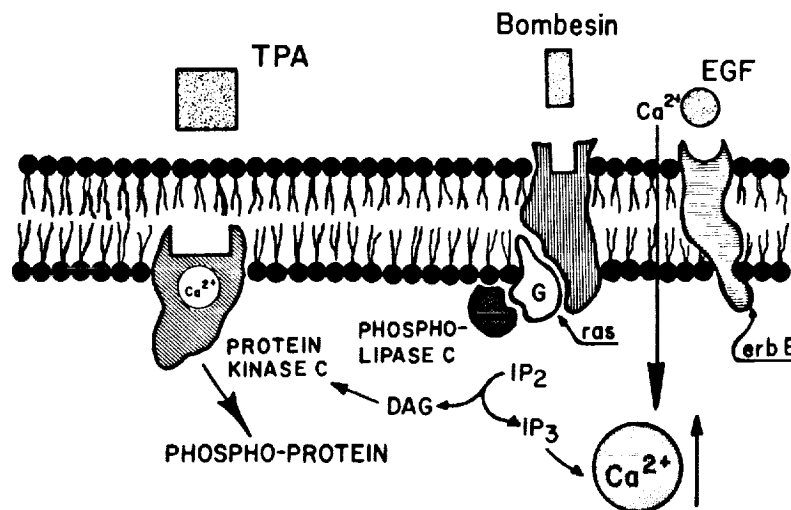


Figure 3. Examples of signal transduction events associated with cell cycling.

complications in the few space-related experiments that have been performed to date, at least some species of higher plants appear to manifest a significantly greater degree of development and differentiation under weightless conditions. Furthermore, the more overt differences between nominal gravity and weightless influences on plant development are manifested at the subcellular level (Lewis and Hughes-Fulford, 1993).

Proliferation of animal cell cultures in microgravity also seems to be different than of ground-based controls although, in general, animal cell proliferation seems to be reduced in the weightless environment. Whether this is a result of altered flow dynamics, mitogen-receptor interactions, events associated with signal transduction, DNA synthesis and chromosome replication, or cytokinesis remains to be determined. Nevertheless, the preliminary observations are both intriguing and hold potential significance to the support of life in the space environment.

Clinostat and sounding rocket experiments have suggested that altered gravity potentially influences the clustering of surface membrane epidermal growth factor (EGF) receptors and subsequent expression of *c-fos*, a gene associated with the entry of cells into the mitotic cycle (Rijken et al., 1990). The mechanism for such a result is unknown although it is interesting that EGF receptor clustering may be a key facet of mitogenesis by this growth factor. Since both chromosome segregation and cytokinesis involve the participation of microtubules, alterations in tubulin assembly in the microgravity environment would be

expected to have dramatic effects on the latter stages of cell division.

One problem that continues to plague space life scientists is the inability to discriminate between true microgravity influences and other, indirect factors that accompany many space missions. In only very few experimental situations have inflight centrifuges accompanied these experiments to produce unit gravity. For instance, one can reasonably question if orbiter and satellite flight experiments are necessarily providing results about reduced gravity or increased radiation. As flight durations increase, the difficulties in discriminating between these two potential influences, and many others, will only become more complex and difficult. Space Station Freedom would be a unique and imperative laboratory for these future experiments since both unit gravity and weightless cells and tissues could be studied simultaneously.

Immune Cell Activities

Of no less importance is the potential influence of reduced gravity on the immune response. In some respects immune cells offer a compelling model system for studies of cell-cell communication. The overall immune response is characterized by a significant degree of cellular interaction, including both suppressor and stimulatory actions, as well as by responses to both direct cell-cell interactions and soluble ligands (lymphokines) that modulate the overall immune response. Furthermore, the concern that

has emerged about the potential of a compromised immune system in reduced gravity, and the myriad of health-related issues that could ensue, makes this an important area of study for the space life sciences.

This area of research has received a great deal of attention and many of the observations have been amply reviewed (Cogoli and Tschopp, 1985; Lewis and Hughes-Fulford, 1993; Sonnenfeld, 1989; Cogoli et al., 1990). Again, the conclusions regarding microgravity and immune cell function are often conflicting and the differences among experimental systems and approaches makes generalization difficult. Nevertheless, there is ample evidence that suggests gravity unloading results in immunologic changes in humans and other vertebrates. In addition to *in vivo* experiments, studies with immune cell suspensions have clearly indicated that the response of the immune system to microgravity may be significant indeed. As discussed by Sonnenfeld (1989), weightlessness, stress, and low-level radiation could all contribute to alterations in the immune response, and what factors are responsible for the measured differences remain to be established.

On one hand, discriminating among the various factors that could influence the immune system remains an important goal for space life sciences in order to more fully describe the basic mechanisms that underlie these altered responses. On the other hand, since a compromised immune system could be a disabling barrier to the long-term health and welfare of humans and other vertebrates in the space environment, understanding changes in immune responses has very practical implications.

Space environment-mediated alterations of the immune system have been known for several years although, until recently, the cellular mechanisms for immune cell changes in reduced gravity have been only sparsely studied. Before the orbiter flights, studies of lymphocytes obtained from astronauts and cosmonauts showed that the mitogen-mediated activation could remain suppressed for days after their return to Earth (Taylor et al., 1986; Gould et al., 1987). However, it has been difficult to discriminate between the possibility that physiological stress-induced changes, rather than a more specific immunological response, was responsible for this immune suppression. A series of benchmark studies, however, illustrated that even lymphocytes in culture could be influenced by reduced gravity, and reduced gravity-mediated changes in the extracellular matrix and/or the plasma membrane have been suggested as a mechanism for this effect (Cogoli et al., 1990).

Although observations with cellular elements of the immune culture would not be expected to necessarily parallel immunological events in the intact host, both *in vivo* and cell culture studies will be

necessary to provide a comprehensive understanding of reduced gravity and the immune cell response. A series of studies on cytokines, at the cellular level, has led to numerous interesting observations. Cytokines are molecular messengers that mediate immune cell-cell communication and orchestrate the overall immune response. In a study after a one-week orbiter flight and upon return of experimental rats to Earth, isolated splenocytes were examined for the relative induction of two lymphokines, interferon-gamma and interleukin-3. While the synthesis and release of interferon-gamma was significantly depressed in comparison to ground-based controls, the production of interleukin-3 was not influenced by the reduced gravity environment (Gould et al., 1987). A recent immune cell study, carried out on a KC-135 parabolic flight, used isolated peritoneal macrophages to show that superoxide (O_2^-) production was four-fold higher in reduced gravity when compared to unit gravity control cultures (Fleming et al., 1991). The altered activity of these inflammatory cells could hold a great deal of significance since superoxide is a major component in macrophage-directed bacterial killing. Although the exact mechanism for the higher production of superoxide in reduced gravity is unknown, it has been speculated that the altered cellular component might well be the Ex-Me-Cy complex (Fleming et al., 1991).

In experiments recently conducted with bone marrow-derived macrophage suspensions during orbiter flight and within 12 hours of reduced gravity, over a three-fold increase in interleukin-1 production was measured in comparison to that measured with ground-based macrophage control cultures (Chapes et al., 1991). In addition, the production of tumor necrosis factor (TNF-alpha), another substance important to inflammatory cell activity, was shown to be significantly stimulated by the space environment (Chapes et al., 1991).

Clearly, the immune system and immune cells themselves are influenced by the microgravity environment, although the degree and direction of the response, and the specific lymphokines involved in the response, appear to vary. Undoubtedly, many new observations will emerge from studies of immune cell function in microgravity, and the information gained may prove to be of significant importance to both basic gravitational biology and space medicine.

SUMMARY

There are many aspects of cell biology and microgravity that go well beyond this necessarily brief assessment. Although there are cells that directly sense gravitational vectors, the primary tenet pre-

sented in this paper is that reduced gravity effects go far beyond the potential role of specialized cells such as plant amyloplasts that aid positioning for plant development in the terrestrial environment. Even though cells may not be able to sense "up" and "down," there are numerous lines of evidence that suggest cells and tissues, for numerous indirect reasons, can be significantly influenced by the microgravity environment. The fact that the influences may be indirect do not detract from their potential importance. The Ex-Me-Cy complex, with its large size and relative instability, is a subcellular structure that could be particularly affected in such an indirect manner. In turn, perturbations of the Ex-Me-Cy complex could influence a significant number of cellular activities and subsequent tissue properties of both plants and animals.

The answers to many of the critical questions in space life sciences, however, will not be solved until more frequent access to the microgravity environment is available, the duration of experimental protocols can be extended to more reasonable lengths, and the proper gravitational force controls are available for experimenters. In this sense, Space Station Freedom is not only important to the future of space life sciences and the biomedical sciences, it is essential.

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