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NASA Grant No. NAG 2-538, The Effect of Spaceflight on Bone Cell Cultures

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A. Summary concepts, hypothesis, aims, methods and significance

Understanding the response of bone to mechanical loading (unloading) is extremely important in defining the means of adaptation of the body to a variety of environmental conditions such as during heightened physical activity or in extended explorations of space or the sea floor. The mechanisms of the adaptive response of bone are not well defined, but undoubtedly they involve changes occurring at the cellular level of bone structure. This proposal has intended to examine the hypothesis that the loading (unloading) response of bone is mediated by specific cells through modifications of their activity, cytoskeletal elements, and/or elaboration of their extracellular matrices. For this purpose, this laboratory has utilized the results of a number of previous studies defining molecular biological, biochemical, morphological, and ultrastructural events of the reproducible mineralization of a primary bone cell (osteoblast) culture system under normal loading (1G gravity level). These data and the culture system then were examined following the use of the cultures in two NASA shuttle flights, STS-59 and STS-63. The cells collected from each of the flights were compared to respective synchronous ground (1G) control cells examined as the flight samples were simultaneously analyzed and to other control cells maintained at 1G until the time of shuttle launch, at which point they were terminated and studied (defined as basal cells). Each of the cell cultures was assayed in terms of metabolic markers; gene expression; synthesis and secretion of collagen and non-collagenous proteins, including certain cytoskeletal components; assembly of collagen into macrostructural arrays; formation of mineral; and interaction of collagen and mineral crystals during calcification of the cultures. The work has utilized a combination of biochemical techniques (radiolabeling, electrophoresis, fluorography, Western and Northern blotting, and light microscopic immunofluorescence) and structural methods (conventional and high voltage electron microscopy, immunocytochemistry, stereomicroscopy, and 3D image reconstruction). The studies have provided new knowledge of aspects of bone cell development and structural regulation, extracellular matrix assembly, and mineralization during spaceflight and under normal gravity. The information has contributed to insights into the means in general by which cells respond and adapt to different conditions of gravity (loading). The data may as well have suggested an underlying basis for the observed loss of bone by vertebrates, including man, in microgravity; and these scientific results may have implications for understanding bone loss following fracture healing and extended periods of inactivity such as during long-term bedrest.

B. Major personnel involved in the project

The individuals who have followed this work throughout its duration include William J. Landis, Ph.D., principal investigator; Louis S. Gerstenfeld, Ph.D., co-investigator; Karen J. Hodgens, B.S., senior laboratory technologist; and Diana Block (Berkery), B.A., laboratory technician. Other individuals have assisted as necessary.

C. Summary results and publications

Summaries of our major results from NASA flights are given below with pertinent references to published work that was an outcome of the experimentation.

1. Osteoblast cell culture development in microgravity. To characterize spaceflight effects on bone cells, our osteoblast cell cultures were inoculated into a number of Cellco CellMax Quad bioreactor units and flown aboard NASA shuttles during STS-59 (April 9-20, 1994) and STS-63 (February 3-11, 1995). The initial experiments in STS-59 utilized cells (~$7x10^6$) attached to 125 mg Cytodex microcarrier beads while the second flight contained the same number of cells but no beads. Respective controls were maintained at 1G during the different missions. Such cultures included cells terminated at the time of the shuttle launch (identified as basal or zero time cartridges or cells) or terminated at the time of the shuttle landing (identified as the control cartridges or cells). Bioreactor cartridge media (DME + 10% FBS) were supplemented with 12.5 μg/ml ascorbate and 10 mM β-glycerophosphate. The first flight included two rails (4 cartridges/rail) flown, each rail having two cartridges containing cells exposed to 5 days of ascorbate supplementation prior to flight and that were committed to the osteoblastic lineage and two with cells exposed to 10 days of ascorbate and were uncommitted to the lineage. Corresponding to the manner of the flight design, two rails with 8 cartridges of either committed or uncommitted control cells and one rail with 4 cartridges of either committed or uncommitted basal cells were maintained. In addition to these bioreactor cartridges, media from the intracapillary and extracapillary spaces of the cartridges, sump bags, and media fraction collections taken at two intervals during the 12 day flight were retrieved from both flight and control units at the termination of the mission, and the basal units were likewise treated on launch, April 9.

Our results from molecular biological, biochemical, and structural analyses of the cells and media have shown that spaceflight exerts demonstrable effects on the cultures. The cells in flight and ground cartridges grew and developed and produced statistically similar glucose and lactate levels. However, while both committed and uncommitted cell flight groups elaborated extracellular matrices principally composed of type I collagen, they did so in lower amounts compared to control counterparts. Correspondingly, type I collagen gene expression appeared to be downregulated by spaceflight, and levels of osteocalcin gene expression were also lower than controls. These data may be highly significant with respect to understanding bone loss following spaceflight and may have implications in our understanding of bone deterioration during prolonged immobility, disuse, or similar conditions at 1G. The data have been presented in WJ Landis et al., ASGSB Bul-

letin 8:50, 1994, and Proc. NASA/AIAA Life Sci. and Space Med. Conf. 1:75-76, 1995. A manuscript by WJ Landis et al., entitled "Spaceflight effects on cultured embryonic chick bone cells," is in press in the Journal of Bone and Mineral Research.

2. Analysis of osteoblast cell cultures from STS-63. As noted above, STS-59 utilized cells attached to microcarrier beads in both control, basal, and flight Cellco bioreactor cartridges. In experiments we conducted in our Children's Hospital laboratory between shuttle missions, we found that our cultures developed more extensively when they were inoculated in the Cellco units without beads. Thus, we flew those cultures without beads (one rail of 4 cartridges) during STS-63 while all other conditions were maintained the same as in STS-59 (except that we flew 2 rails of 8 cartridges in that mission; more space in the shuttle mid-deck locker was available to us during STS-59 than STS-63). We have compared some control, basal, and flight cartridges ultrastructurally and found all grew more extensively, and over a shorter flight time (for the control and flight cartridges; basal cartridges had already been terminated), than their counterparts in STS-59. Importantly, the amount of matrix in the flight cartridges was less than that in the controls in this mission, as we had observed in STS-59. For added comparison with other results from STS-59, we analyzed gene levels of expression for collagen, osteocalcin, and osteopontin in flight, basal, and control cartridges to determine possible correspondence with the reduced flight matrix quantities. Each of these determinants was down-regulated in spaceflight, the data for collagen and osteocalcin supporting the results found in STS-59. Finally, osteocalcin, osteopontin, and bone sialoprotein were identified in both flight and control cartridges by immunocytochemistry and electron microscopy, but each of these proteins appeared with reduced immunoreactivity following flight compared to control. The information obtained from these studies corroborates results from STS-59 that spaceflight mediates adaptation of cultured bone cells, in part through changes in both collagen and non-collagenous proteins and their gene expression. The data have been published in WJ Landis et al., ASGSB Bulletin 11:71, 1997; and WJ Landis, ASGSB Bulletin 12:4, 1998. A manuscript by WJ Landis, entitled "An overview of vertebrate mineralization with emphasis on collagen-mineral interaction," is in press in the American Society for Gravitational and Space Biology Bulletin.

D. Inventions and Patents

No inventions or patents were developed in association with this grant.

E. Property

There were no items of equipment, supplies, or the like purchased under this grant that had a value of $5000 or more.