EFFECT OF GRAVITY ON MONOCYTE DIFFERENTIATION

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ABSTRACT

Early space flight experiments have shown that microgravity suppresses the immune response in humans, especially in T- cells activation and monocyte differentiation. Protein Kinase C (PKC) is a key protein controlling growth and differentiation of monocytes into macrophages. Previous studies by Hatton et al. showed that the distribution, cellular quantity and kinetics of translocation of PKC are altered in microgravity.

We have conducted studies of PKC activation using phorbol 12,13-dibutyrate (PDBu) under normal and altered gravity on our PKINASE experiment on ISS. Examination of differentiation of monocytes under real microgravity and normal gravity show significant changes in the molecular function gene expression during early signalling as well as in expression of differentiation of the monocyte. PDBu causes differentiation of the monocyte and results in adhesion of the cells within 24 hours. Cumulatively, these results suggest that activation of early signal transduction is regulated in part by gravity.

In true microgravity (μg) we see significant changes in gene expression of the monocyte between activation of the μg samples and the 1g in-flight samples. These changes in gene expression suggest a role of gravity in regulation of immune function in human monoctytes. (Supported by NASA NCC 2-1361 and many thanks to the teams of ESA, ESTEC, ESA-Moscow, IBMP, Baikonur labs and the Cosmonauts for making this experiment possible).

Introduction: Early studies in microgravity have shown changes in PKC signal transduction in U937 monocytes (1-3). Activation of the monocyte by PKC results in differentiation of the monocyte into a macrophage and adhesion. In these ISS studies, we analysed the induction of gene expression in non-treated cells (NT), cells activated by PDBu for 3 hr and 24 hours. We examined the differences in molecular function in 1.g and μg. The molecular functions of a gene product are the jobs that it does or the "abilities" that it has. These may include transporting things around, binding to things, holding things together and changing one thing into another. This is different from the biological processes the gene product is involved in, which involve more than one activity.

One way to understand this is to consider the analogy of a company or organization. Individuals (gene products) have different abilities or tasks (functions) and they work together to achieve different goals (processes). It is easy to confuse a job title (gene product name) with a function; for example, 'secretarial activity' may seem like a valid function because you have a good conceptual idea of what a secretary does. However, in different companies, secretaries might do different things. One secretary might have the functions 'typing', 'answering phone' and 'making coffee', while another might have these functions and additionally 'photocopying'. In the Gene Ontology, a function should be unambiguous and it should mean the same thing no matter what species you are dealing with. (see http://www.geneontology.org/GO.function.guidelines.shtml).

Hardware: Cells were grown in floating piston hardware made by Kaiser Italia**.** Cells were placed into the hardware in Baikonur, Kazakhstan prior to the launch of the Soyuz taxi flight to the International Space Station (ISS). Once on ISS, the cells were placed into the KUBIC incubator for 3 hours prior to activation. Cells were then activated and placed into 1.g centrifuge or μg conditions inside the incubator.

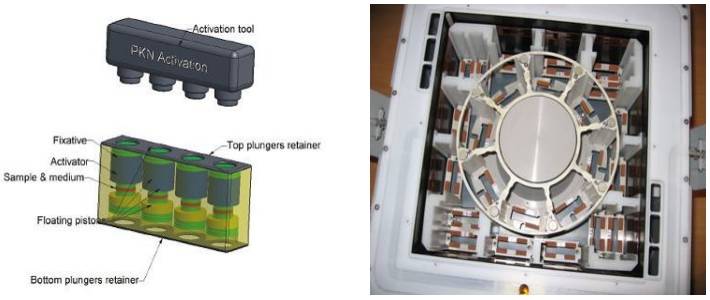

Fig.1 the flight hardware and the Kubic incubator

METHODS: *RNA Cleanup and processing*

Cell total RNA was stabilized by RNA*later* (Qiagen, Valencia, California). RNA was isolated using Trizol® followed by RNeasy® Micro kit (Qiagen, Valencia, California). RNA concentration and purity was determined from measuring absorbance at 260 nm and 280 nm and 0.3 μg total RNA was run on a 1% denaturing gel, or 100 ng total RNA was loaded on the 2100 Bioanalyzer (Agilent, Palo Alto, California) to verify RNA integrity.

Microarray sample preparation:
For microarrays analysis, the RNA was amplified using the Ambion MessageAmp II-Biotin Enhanced kit (Ambion, TX) and gene expression was determined using Affymetrix U133 plus 2.0 arrays (n=3) which allows complete coverage of the human genome plus 6,500 additional genes for total analysis of over 47,000 transcripts as previously described (*4*). By using triplicate independent biological samples, we are able to determine statistical changes in expression and find the genes that are dramatically induced during differentiation

Data analysis and gene molecular function:
Prior to analysis, several internal parameters from each Affymetrix GeneChip® were checked to verify sample and array quality such as noise and background levels, present calls, and 3'/5' ratios. Background subtraction and image processing in Affymetrix GeneChip Operating Software (GCOS), data normalization was performed in GeneSpringGX® 7.3 (Silicon Genetics, Redwood City, CA). Significant differential gene expression was identified after scaling each gene to its corresponding signal in the 0 hr baseline to derive expression ratios. Only genes flagged with a present call in at least one array were considered for subsequent analysis. To focus on genes normally induced by PDBu a 2-fold upregulation cut-off at 1.g 3 and 24-hr was established. The cut-off for significance was set at $P \leq 0.05$, and all parametric tests were performed on the logarithm of expression ratios.

RESULTS and DISCUSSION: When we add PDBu in normal gravity the U937 cells activate PKC, resulting in adhesion and differentiation during the following 24-36 hours. In Fig. 2 we see the effect of PKC activation on adhesion of the U937 monocytes cells at 0, 24 and 36 hours.

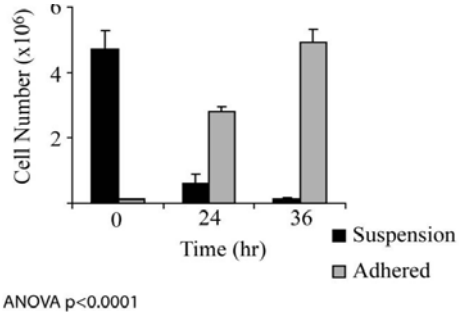

Fig. 2. PKC mediated adhesion in U937

The examination of the gene array data (Fig. 3) shows a significant decrease in the change in the expression of binding, catalytic activity, activation of signal transduction, motor activity, structural molecule and transporter activity three hours after PDBu treatment in ug. These data suggest that the early signal transduction and enzyme activity requires gravity for normal differentiation of the monocyte.

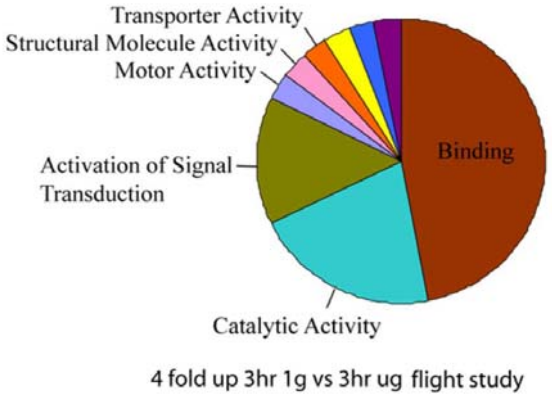

Fig.3 Molecular function in U937 cells

Twenty-four hours after activation with PDBu, we found other significant reductions of expression of binding, catalytic activity, activation of signal transduction, regulation of transcription, and enzyme regulation

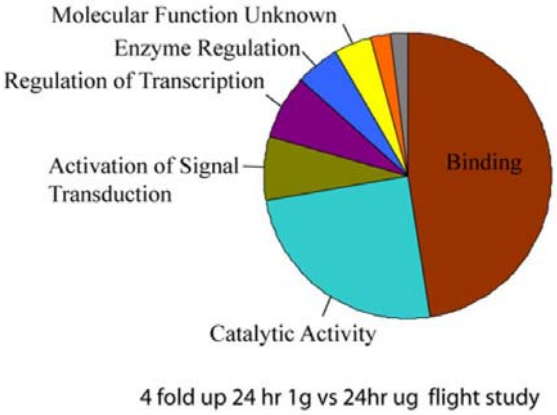

Fig. 4: Molecular function of U937 Cells 24 hrs

The 24-hour data also reflect the lack of binding, enzyme activity and signal transduction in the monocytes under microgravity conditions. Taken together, this preliminary data from PKINASE suggest that gravity plays a role in normal differentiation and gene expression of the monocyte into a macrophage.

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