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Cell Biology in Space: Basic research and potential applications

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The purpose of this paper is to present an overview of our work conducted in space and on ground in simulated microgravity the past three decades. When, 1976, I proposed to the European Agency to study the effect of weightlessness on cell differentiation, very few scientists believed that the absence of gravity might have an influence on the biological processes taking place in the micro-cosmos of single cells. Indeed, the differences between the density of cell organelles and that of the cytoplasm together with the flexibility of the cytoskeleton strongly support the notion that gravity may directly interfere with cellular processes.

As model for our studies we have selected the mitogenic activation *in vitro* of T cells. Such model permits to investigate crucial events occurring during the cellular immune response as well as a differentiation process in which resting T lymphocytes are triggered to produce lymphokines (in particular interferon-gamma, interleukin-2 and its receptor) and to proliferate. Our interest focused both on the immune system of humans in space and on signal transduction in differentiating cells.

Experiment with T cells

In November 1983 the Spacelab module was flown in the payload bay of the shuttle Columbia in the Spacelab I mission. In an incubator manufactured in our workshop we determined the mitotic index of T cells activated and chemically fixed in flight. Activation of cells was determined by measuring the tritiated thymidine incorporated into DNA. There was a dramatic loss of activity (more than 95%) compared to identical samples kept on the ground (Cogoli *et al.*, 1984). That discovery triggered the interest of the scientific community and several experiments were performed by us and others in space and on ground in order to clarify how gravity could interfere with the signal transduction and differentiation mechanisms in T cells.

In all following studies in space we used a reference centrifuge generating artificial I g gravity.

The results from Spacelab I were confirmed in 1985 in the Spacelab D1 mission.

A new approach was adopted in a study performed in 1991 in Spacelab SLS1 (there had been a long interruption of the shuttle flights after the catastrophe of Challenger in 1986). T lymphocytes (known to be adhesion-independent cells) were coated to microcarrier beads. To our great surprise the depression of mitogenic activation observed in resuspended cells was not observed in coated cells (Bechler *et al.*, 1992). On the same mission, by means of a "multi-g centrifuge" we observed that there must be a thresholf of sensitivity between 0 and 0.6 g (Cogoli *et al.*, 1993).

In the last 20 years we have performed in collaboration with other laboratories (in Sassari, Rome, Florence, L'Aquila, S. Francisco and Berlin) several experiments in Spacelab, sounding rockets, and stratospheric balloons. Such experiments were accompanied by extensive investigations conducted in the ground laboratory in fast rotating bi- and three-dimensional clinostats.

In the Spacelab IML 2 mission, flown in 1994, a centrifuge microscope developed by the German Space Agency, DARA GmbH, based on plans proposed by Briegleb and Hemmersbach (Friedrich *et al.*, 1996) permitted to record the movements and interactions of lymphocytes in microgravity. The experiment addressed a crucial question related to the loss of T cell activation in space, namely whether the cells are capable of autonomous movements and of establishing cell-cell contacts under 0 g conditions. In fact, T cell activation requires tight contacts between each other as well between T cells and monocytes as antigen-presenting cells. We were able to see that cells display autonomous movements and interactions in 0 g (Cogoli-Greuter *et al.*, 1996). We investigated the structure of the cytoskeleton of the intermediate filaments of vimentin in Jurkat cells (a cell line derived from human T cells) by immunofluorescence technique on the sounding rocket MAXUS 1B. There was a significant higher formation of large bundles of filaments already 30 s after exposure to 0 g thus showing that the cytoskeleton undergoes important and immediate changes in microgravity (Cogoli-Greuter *et al.*, 1998).

The structure of F-actin filaments was investigated in 2006 in a facility called KUBIK on board of the International Space Station, ISS. Again important differences between the distribution of actin between 1 g and 0g in the macrophage cell line J-111 were observed (Cogoli-Greuter *et al.*, manuscript in preparation).

The data obtained in space as well as in clinostats can be summarized as follows: (i) already 30 seconds after

exposure to microgravity there are significant changes in the structure of the cytoskeleton; (ii) cell-cell interactions and cell movements are taking place also in a weightless environment; (iii) the genetic expression of important proteins and factors involved in T cell activation is significantly depressed; (iv) protein kinase A (PKA), in addition to protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) pathways, plays a role in early T cell activation and induction of interleukin-2 (IL-2), interleukin-2 receptor alpha subunit (IL-2Rα) and interferon-gamma (IFN-γ) gene expression (Hughes-Fulford *et al.*, 2005); (v) the data show that the downregulation of early genes regulated primarily by transcription factors NF-κ B, CREB, ELK and STAT contribute to T cell dysfunction in a microgravity environment; (vi) the fact that phosphorylation of the linker of activation in T cells (LAT) is not down-regulated in simulated microgravity indicates that cholesterol-rich lipid rafts are not involved in the down-regulation of the transcription factors (Boonyaratanakornkit *et al.*, 2005), (vii) T cells undergo apoptosis in 0g conditions (Maccarrone *et al.*, 2003).
In parallel with the investigations on signal transduction we have also studied the activation of T cells from astronauts prior to, in and post flight. The data confirm early findings of US and Russian scientists who reported a depression of the mitogenic activation in lymphocytes in blood samples taken immediately after flight. The values return to the baseline within one week after landing. It is still not clear whether such depression is caused by the exposure of the human body to weightlessness or rather to the physical and psychological stress of the space flight. The study of the impact on the immune system of astronauts that has been rather neglected in the past is gaining now high priority in the perspective of long duration missions to the planet Mars. A comprehensive review of our work with cells of the immune system has been published recently, (Cogoli and Cogoli-Greuter 2005).

Potential applications

The interesting and somehow unexpected behaviour of cells in space induced us to develop sophisticated instruments for biotechnological processes in space laboratory. A first step was the development ob a bioreactor that was flown on three space missions (Spacelab IML-2, STS-76 in 1996 and STS-107, that ended with the catastrophe of Columbia in 2003) with yeast cells of *Saccharomyces cerevisiae*. The analysis of the electron micrograph from the experiment on IML-2 showed a significant difference in the distribution of the bud scars (remaining after the separation of a daughter cell) between 1 g in flight controls and 0 g samples: while a majority of the scars show bipolar distribution at 1 g, there is a significant higher random distribution at 0 g, Walther *et al.* (1996). These data suggest that there are remarked changes in the intracellular structure.
In an effort to take profit of microgravity for biomedical and biotechnological applications we started a series of investigations conducted in the three-dimensional clinostat, called also random positioning machine, RPM.
One project is dedicated to the study of osteoporosis by means of a newly developed bone resorption assay: The experimental model consisted of the human bone marrow derived FLG 29.1 cell line, previously characterized as an osteoclastic precursor model which adhered to bone slices on which a rut was scraped with a scalpel. The profile of the rut was measured after 3 days of incubation in the RPM. There was a clear and measurable resorption of the bone under simulated 0 g conditions, whereas no change was detected in the control samples (Monici *et al.*, 2006). We plan to use such model to tests drugs and food supplements that could prevent osteoporosis.
We studied the behaviour of epithelial cells in microgravity for two reasons. One is to better understand how wound healing in space proceeds. In fact, it is known since the early time of human space flight that wound healing is retarded in 0 g. The other reason is to investigate angiogenesis in microgravity in order to explore whether the production of artificial tissue (e.g. blood vessels) could be improved in zero g. The formation of a tube-like structure by epithelial cells was observed in the RPM (Infanger *et al.*, 2006). A tubular structure (like a lumen) with a length of 1.8 mm and an artificial endothelium layer along the extracellular matrix were formed after 72 h culture under simulated microgravity.
Spheroids of tumour cells are useful cell aggregates that can be regarded as a tumour model suitable for the study of anti-tumoral drugs. We have investigated the formation and properties of spheroids of thyroid carcinoma cells grown in the RPM (Grimm *et al.*, 2002).
In conclusion, these findings suggest that the alterations of single cell behavior observed in the absence of gravity may be exploited for biotechnological and biomedical applications. Examples from our own research in clinostats are (i) the study of osteoporosis in an in vitro model based on bone slices and osteoclasts; (ii) the formation of multicellular tumor spheroids as a model of solid tumors; (iii) the formation of cartilage tissue from chondrocytes.

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