From Cell Biology to Biotechnology in Space

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In this article I discuss the main results of our research in space biology from the simple early investigations with human lymphocytes in the early eighties until the projects in tissue engineering of the next decade on the international space station ISS. The discovery that T lymphocyte activation is nearly totally depressed in vitro in 0 g conditions showed that mammalian single cells are sensitive to the gravitational environment. Such finding had important implications in basic research, medicine and biotechnology. Low gravity can be used as a tool to investigate complicated and still obscure biological process from a new perspective not available to earth-bound laboratories. Low gravity may also favor certain bioprocesses involving the growth of tissues and thus lead to commercial and medical applications. However, shortage of crew time and of other resources, lack of sophisticated instrumentation, safety constraints pose serious limits to biological endeavors in space laboratories.

The purpose of this article is to describe the progress of twenty years of research in space conducted by our laboratory, starting from simple experiments with single cells and developing to potential biotechnological application.

This is interesting because at the beginning there was a broad skepticism on the value of biological investigations in low-g conditions. The surprising finding that signal transduction in T lymphocytes is dramatically changed in zero-g awoke the interest of the scientific community and indicated that important biological events can be influenced by gravity within single cells.

In general, the development of space biology can be subdivided into three phases. In the first one (from the early seventies to the mid eighties) living system were studied at random to look for detectable effects of the space environment. In the second phase, which lasted until the mid-nineties, several important effects on cellular mechanisms were discovered and characterized. A comprehensive review of the most important biological experiments of these phases has been presented in (Moore and Cogoli, 1996). The third phase is at its beginning and consists of the use of 0 g as a tool for basic research, for medicine and for biotechnology.

Our first experiment was carried out in August 1983 with human embryonic kidney cells in a simple incubator installed in the flight deck of the Space Shuttle Challenger. From then on we developed instruments and technologies that are now leading to the definition of bioreactors for tissue engineering on the International Space Station (ISS). Most of our studies with single cells were dedicated to the behavior of human lymphocytes purified from the blood of healthy donors prior to the flight or cultured in whole-blood samples taken in flight from crew members. For this reason I describe first the results obtained with human lymphocytes. A description of our space bioreactor will follow. Finally the perspectives of biotechnology in space are discussed. An overview of the experiments is given in Table 1.

T Lymphocytes

The study of the activation of T lymphocytes in space turned out to be one of the most intriguing story of space biology. Due to their role in cellular immunity and to the complexity of their activation mechanism, T cells are since decades objects of extensive investigations worldwide. In the early seventies Russian scientists were the firsts to report that the activation by mitogens of lymphocytes from astronauts was depressed after flight. Similar results were reported a little later by US investigators (see, for reviews Cogoli and Cogoli-Greuter, 1997, Gmünder and Cogoli, 1996). This may point to a higher risk of infection during and after space flight. To study the problem in more detail it was suggested to test lymphocyte activation in cell cultures in space. Three lines of experiments were conducted: *in vitro*, *ex vivo* and *in vivo* studies.

Ex vivo experiments are based on blood samples drawn from space crew members prior to, during and after flight which are diluted with culture medium and incubated in the presence of a T lymphocyte activator called mitogen (usually concanavalin A). *In vivo* studies consist of the application of antigens to the skin of space crew members in order to determine the delayed

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Table 1. Space experiments carried out by the Space Biology group of the ETH Zurich

Mission	Year	Experiment / Results
STS-8	1983	Human embryonic kidney cells: Cells of potential interest for biotechnological applications (producer of tissue plasminogen activator) adhere normally to microcarrier beads in 0 g
STS-9/Spacelab-1	1983	Human lymphocytes: Activation of peripheral blood lymphocytes with the T cell specific mitogen con A is strongly inhibited at 0 g
STS-61-A/Spacelab D1	1985	2 experiments with human lymphocytes: The data from Spacelab 1 were confirmed and further supported by the use of a 1 g reference centrifuge in flight. Blood samples from 4 astronauts were cultured in flight.
STS-40/Spacelab SLS-1	1991	2 experiments with human lymphocytes: The experiments of D 1 were repeated with an update protocol. Biochemical analysis indicated that depression of IL-2 and IL-2R production may be one of the causes of the depression of activation discovered in Spacelab 1.
STS-42/Spacelab IML-1	1992	3 experiments with hybridoma, hamster kidney, Friend cells. No dramatic difference in cell proliferation, differentiation and antibdy secretion observed. The DCCS, a "minibioreactor" based on osmotic pump was tested.
STS-65/Spacelab IML-2	1994	3 experiments: 2 with human lymphocytes, space bioreactor. White blood cells are capable of autonomous movements and of cell aggregation as well as cell-cell contacts in 0 g. A sophisticated bioreactor supported well the culture of yeast cells of *Saccharomyces cerevisiae*
STS-76/SSM-3	1996	Space bioreactor: An improved version of the instrument flown in IML-2 was again usde to culture yeast cells.
STS-81/SSM-5	1997	Human lymphocytes: The technology for preservation of viable frozen cells was tested in space.
MIR missions 7, 8, 9	1988, '89, '90	3 experiments on the immune response of cosmonauts on long-duration flights. The stress of space flight depresses the response of T cells measured after application of 6 different antigens on the forearm skin.
Biosatellite 9	1989	Test of DCCS with plant protoplasts
MASER 3, 4	1989, '90	Human lymphocytes: Binding of Con A occurs normally, patching is retarded at 0 g. This indicates possible alteration of the cytoskeleton.
MAXUS 1, 1b, 2	1991, '92, '95	Jurkat T cell line: Cells display autonomous movements, microtubuli (vimentin) structure is altered after 30 sec exposure to 0 g.
STS-107	2001	2 experiments: genetic expression in purified T lymphocytes, bioreactor with yeast cells, osmotic and heat stress in 0 g.
MASER 9	2001	2 experiments: early genetic expression in T lymphocytes, cytoskeleton and genetic expression in human chondrocytes (MAP project)
STS-117/R2?	2002?	Chondrocytes: formation of cartilage in 0 g (MAP project)
STS, ISS	TBD	Chondrocytes: formation of cartilage in 0 g (MAP project)

hypersensitivity, i.e. the specific response of T lymphocytes, prior to, during and after flight to a number of antigens (delayed-type hypersensitivity test or skin test). In vitro experiments are based on immune cells isolated from the peripheral blood of healthy donors (not necessarily astronauts) a few hours before the experiment is started, either in the space or in the ground laboratory, and cultured in a standard culture medium in the presence of a mitogen.

T lymphocytes from human peripheral blood may be activated in vitro by several substances of different origin, the mitogens, which are able to trigger the events occurring in vivo following exposure to an antigen. The activation of T cells with mitogens is, therefore, a good model to simulate in vitro this key aspect of the immune response. Concanavalin A is a widely used mitogen. The activation process consists of three phases: (i) recognition of the antigen/mitogen; (ii) cell-cell interaction and exchange of signals between T cells (secreting interleukin-2) and accessory cells, usually monocytes (secreting interleukin-1 and interacting wia their ligand B7 with the CD28 receptor on the T cell); (iii) expression of interleukin-2 receptor and recognition of autocrine interleukin-2 by T cells.

The objective of ex vivo and in vivo studies is to assess the efficiency of the immune system in humans exposed to the stress of space flight. The objective of the in vitro experiments is to investigate the biological mechanism of T cell activation under the influence of gravitational changes. The in vitro experiments in low-g have contributed to understand certain aspects of signal transduction in T cells. Studies ex vivo and in vivo on the immune cells and on the delayed hypersensitivity of astronauts in Spacelab and in MIR space station, respectively, have helped to distinguish between effects of gravity and effects of physical and psychological stress.

Ex vivo experiments

This is a typical case in which an in vitro cellular response to an inducer is used to measure the efficiency of the specific immune response although its clinical significance is not clear and it is difficult to correlate the changes of this parameter with a real pathological situation. More than 50% of the 129 subjects tested after Russian and US missions in the last 20 years showed depression. An accurate comparison of the data obtained by different investigators is hampered by the fact that the experimental protocols are very heterogeneous.

Mitogenic activation of astronauts' blood samples was conducted in space for the first time by our laboratory in Spacelab D-1 in 1985, three subjects showed depression (20-50%) in flight as well as immediately after recovery. Baseline values were reached within 7 days after landing. Conversely, four subjects tested in Spacelab SLS-1 1991, did not show significant changes of the mitogenic response neither in flight nor after flight. Nevertheless, the remarkable depression of the mitogenic response in astronauts is well documented. In all cases measured so far, recovery occurred within two weeks after landing. No health consequences have been reported and the activation values returned to the pre-flight baseline within one or two weeks.

In vivo experiments

The delayed-type hypersensitivity test used aboard the Russian space station MIR was based on a modified Multitest Mrieux which is commercially available and is designed to test cell mediated immunity involving T lymphocytes. The test consists of the intradermal application of antigen and toxin preparations. A positive response consists of a pink-colored induration of the skin appearing 24-48 h after the application of the antigens and similar to that observed after the bite of an insect. The term delayed-type is used to distinguish it from the immediate-type hypersensitivity, which occurs within minutes after exposure to the antigen. A delayed hypersensitivity test was carried out for the first time in space on shuttle astronauts by US investigators. A weak trend to lower cell mediate immunity at the end of short Space Shuttle missions (four days duration, three subjects tested) and a more pronounced decrease of the delayed-type hypersensitivity responses at the end of longer missions (five and ten day duration, three and four subjects tested, respectively) were noted. It was concluded that the immune system is most depressed between flight day five and ten. In a collaborative project between our laboratory and the Institute of Biomedical Problems in Moscow, cell mediated immunity was determined on astronauts on long duration missions lasting between 132 and 177 days. It was seen that after particularly stressing and life threatening operations (like an unforeseen extravehicular activity to perform urgent repairs) the response was dramatically lower than the baseline.

Individuals exhibiting low in-flight and/or post-flight score reactions may be afflicted with reduced cell mediated immunity at the time of the test. The data from the *in vivo* and *ex vivo* studies strongly support the conclusion that it is the stress of space flight and not low-*g per se* the main cause of the depression of certain immunological parameters observed in a majority of the astronauts.

In vitro experiments

The firsts to study cultures of lymphocytes in space

were Hungarian and Soviet investigators who discovered that the production of interferon-α induced by polynucleotides in human lymphocytes cultured on the soviet spaceship Salyut 7 was increased by 500% compared to the ground controls. Activation by concanavalin A was carried out for the first time in space in our experiment in Spacelab-1 in 1983. Surprisingly the inhibition of activation was 93% despite the fact that the cells formed aggregates in low-*g* (Cogoli et al., 1984). The results were confirmed with a series of experiments performed in Biorack in Spacelab D-1, IML-2 and in Spacelab SLS-1, and in sounding rockets (Pippia et al., 1996, Cogoli-Greuter et al., 1996). The data obtained in low-*g* shed some light on the still obscure mechanism of T cell activation. In summary, it was seen that (i) the delivery of the first activation signal, namely the attachment of the mitogen to the cell membrane occurs normally at 0 *g*; (ii) the second signal (interleukin-1 and/or cell-cell interaction via B7/ CD28) is delivered normally by the monocytes as accessory cells; (iii) the third signal (interleukin-2) is secreted normally by the T lymphocytes in their initial activation phase; (iv) cell-cell interactions occur normally at 0 *g*. The interleukin-2 receptor (that has to be expressed in the third activation step), however, is not inserted on the membrane of the T cells. Moreover, changes in the structure of the cytoskeleton (e.g. vimentin microfilaments) are observed few seconds after exposure to 0 *g*. Finally, simulations of low-gravity conditions conducted on ground with the three-dimensional clinostat showed that the genetic expression of IL-2 and its receptor are significantly depressed (Walther et al., 1998).

In conclusion, the lymphocyte case is a very good example of how low-*g* is used as a tool to study earthbound problems like the relationship between physical and psychological stress (neuroendocrine system) and the immune system as well as the complex mechanism of signal transduction in immune cells.

Tissue Engineering and Bioreactors

Another important activity of our group is the development of sophisticated instrumentation for biology and biotechnology in space. The first instrument was a tissue culture incubator flown with human embryonic kidney cells in the flight deck of the space shuttle in flight STS-8 in 1983. Identical models were flown in Spacelab-1 and Spacelab SLS-1 with human lymphocytes.

The most interesting instrument is a bioreactor developed in collaboration with MECANEX, S.A., Nyon, and the Institute of Microtechnology of the University of Neuchâtel (Walther et al., 1994, 1996, 1999). The introduction of microsensors, of a new pH control system based on the electrolysis of water instead of the traditional neutralization of acidity with NaOH and of piezoelectric micropump for fresh medium supply opened

new ways to bioreactor technology.

The instrument flew with yeast cells in Spacelab IML-2 and in Shuttle flight STS-76. An improved version will fly in 2001 on STS-107. The idea behind a bioreactor for space laboratories is the development of a modular bioreactor system for tissue engineering in low gravity. A project has been recently selected by ESA within the application and commercialization program of the *International Space Station. Team* members from academic institutions are A. Bader, Hannover; S. Ambesi, Udine; P. Bruckner, Münster; R. Portner, Hamburg; A. Cogoli and I. Walther, Zurich; W. Müller is the industrial partner from Sulzer Medica, Winterthur. The objectives of the project are: to develop procedures of *in vitro* organogenesis of pancreatic islets, thyroid tissue, liver, vessels and cartilage; to study the mechanism of organogenesis in low-*g*; to define the requirements of a modular space bioreactor for medically relevant organ-like structures; to set up procedures for the production of implants for medical applications. It is believed that low-*g* may contribute in two aspects to progress in this field. First as a useful and non-invasive tool to study important and still obscure biological events like signal transduction, gene expression, and cell proliferation. Second, low-*g* may favor the mass production of cells by obtaining higher cell densities per unit culture volume as well as a smooth cell-cell aggregation and three-dimension organogenesis in the absence of sedimentation and shear forces. Preliminary tests in the random positioning machine will be followed by flights in space in 2001-03.

Weightlessness and Biology

The transition of a cell culture from the terrestrial 1 *g* conditions to 0 *g* in space has a dramatic impact on the environment of the cells. Although the phenomena observed at the cellular level are not yet fully explained it is clear that the loss of buoyancy and of hydraulic pressure in a weightless fluid must have an influence. For the same reason the fluid distribution (lymph and blood) in the body of an astronaut is also affected. One of the consequences is an increase of the viscosity of the blood that changes the environment of the circulating leukocytes.

The most important changes are the loss of sedimentation, of density-driven convection and of hydraulic pressure. For a cell immersed in a fluid, as it is the case in a culture, this is a completely new situation. First, at 1 *g* mammalian cells sediment within a few minutes to the bottom of the flask where many of them may spread and adhere. At 0 *g*, instead, cells remain resuspended. This is a change from a two- to a three-dimension environment and has a remarkable impact on cell interactions, on cell movements and - due to the lack of a substratum on which to spread and adhere - on cell shape. Second, density-driven convection (due to changes of the concentration of nutrients

and waste products in the medium) does not occur at 0 *g* thus preventing mechanical diffusion. Thermodynamic diffusion is not affected, however. Third, the cytoskeleton is a rather flexible structure resembling more the web of a spider rather than a bone skeleton. Cell organelles have densities, which are significantly higher than that of the cytoplasm. Thus, due to their mass, organelles exert at 1 *g* a certain pressure on the microfilaments of the cytoskeleton. Important intracellular processes, like signal transduction, may be affected when such interactions disappear at 0 *g*. Fourth, a new convection, predicted at the beginning of this century by Marangoni, and not detectable at 1 *g* becomes relevant in low-*g*. The lack of buoyancy prevents gas bubbles (e.g. of CO_2 developed by the metabolism of living cells) to rise to the surface of a culture, thus favoring the formation of larger bubbles in the middle of the liquid phase rather than a separation of the liquid and gas phases.

An important question in this context is "can the data from measurements *in vitro* be extrapolated to the *in vivo* situation? The answer to this question is that, although the experiments *in vitro* as well as those *ex vivo* show a depression of the function of T lymphocytes, the two phenomena have different origins. The effect on cell cultures is likely a direct effect of low-*g*, that on the lymphocytes from astronauts is related to the effect of stress on the neuroendocrine system.

At difference with aviation and astronautics the results and impact of the findings of space biology are not well known to the majority of the scientific community. Main reasons are the limited access to space laboratories and the difficulty to repeat the experiments to confirm the results and to increase their statistical significance. Nevertheless, the data collected so far confirm the scientific, technological and biomedical relevance of space biology. Some of the problems preventing a large community of scientists from conducting experiments in space are outlined here.

First, the access to space is restricted. Only a little number of projects can be accommodated in a Spacelab flight. The consequence is that the statistical significance of the data is sometimes questionable and the reproducibility of important results is difficult to verify by independent team. Second, the resources available in a space laboratory are very limited. Energy, weight and volume of the payload as well as crew time have to be shared among several users from different disciplines as material and fluid sciences, medicine and biology. This means a significant restriction of the manipulations, of the analytical procedures (as microscopic and biochemical determination) and of the controlled storage/stowage of biological samples in orbit. Another disturbing limitation is the so-called late access time, i.e. the latest time at which biological samples can be delivered for installation on board. This time ranges between 15 and 25 h before launch. Several living probes must undergo special treatment

in order to be viable for the processing in orbit. The consequence is that the experimental protocols are less sophisticated and comprehensive than those of equivalent investigations on Earth. There is a consensus in the scientific community today, that centrifuges providing 1 *g* in space are necessary to control for all those environmental elements (as vibrations, accelerations, temperature fluctuations and, most important, cosmic radiation) typical of space flight. While Biorack was fitted with a 1 *g* centrifuge, most of the other experiments performed in other flights of the shuttle lacked of such control.

Third, the safety of the astronauts requires severe acceptance criteria for instruments and biological materials on board. Fourth, failures due to instrument malfunctions, breakdown of resources, crew errors may even cause the total loss of an investigation prepared for years, often without an opportunity to re-fly.

What is It Good for?

There are at least four good reasons that are motivating the efforts and the patience of space biologists. One is the scientific curiosity to expose living systems to conditions (low-*g* and cosmic radiation) never experienced before throughout evolution. The unexpected and important results of several experiments show that even very simple organisms display dramatic changes in low-*g*. In this context, low-*g* can be considered as a new tool to study complex biological mechanisms from a new perspective. For example, in the case of cell cultures the transition from 1 *g* to 0 *g* changes the geometry of the system from two-dimensional to three-dimensional. Most living systems are thermodynamically very complicated non-equilibrium systems. Therefore, they may follow interesting bifurcations. Low-*g* may favor a new path, which is not known at 1 *g*. The study of such a path contributes to the clarification of unknown biological processes. As low-*g* and cosmic radiation are not reproducible on Earth, the only way to perform this research is to go to space. Simulations in devices like clinostats are a useful and necessary complement, but not a replacement for space.

Another reason is to study specific physiological functions at the cellular level, either *in vitro*, i.e. in a test tube, or *ex vivo*, i.e. in cells drawn from tests subjects exposed to the conditions of space flight. Examples are the study of the immune system with peripheral blood leukocytes or of the bone system with chondrocytes and osteoblasts. Such studies have contributed, for instance, to the study of the effect of physical and psychological stress on the human immune system. This is a very interesting topic of neuroimmunology, a young discipline of growing importance in today's hectic life.

A third reason is the technological return of space biology. The constraints of space flight result in high-

tech challenges in the application of analytical techniques and in the development of flight instrumentation. Examples are the development of microtechnology for sensors and regulation systems. In fact the limits of weight and volume in orbit do not permit to work with the aliquots biologists are used to on ground. An example of sophisticated instrumentation is the development of a space bioreactor installed in Biorack (see above). In addition, basic research with single cells in space may show new perspectives in biotechnology and biochemical bioengineering. There are great expectations that 0 *g* conditions may be optimal for the development of artificial tissues and organs. Tissue engineering could become one of the modern and growing technologies, which will profit mostly of the biological facilities that will be installed on the ISS.

Finally, the last but not least reason is the exploration of space. This includes trips to an earth orbit as well to the planets of the Solar system and, in a far future, to other planetary systems. It is important that the adaptation of the physiological functions of humans and other mammals as well as of other organisms like plants, invertebrates and microbes are investigated and clarified. It was and it will be an irresistible drive of our mankind to explore first all continents of the planet Earth and, later, any accessible site of the Universe as soon as the required technology becomes available. Space exploration includes also the search for extraterrestrial life. The study of terrestrial life out of the terrestrial environment will contribute to the identification and understanding of alien forms of life.

Acknowledgements

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