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Space Biology Group

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Basic Research, Biotechnology, Tissue Engineering, and Instrument Development

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Abstract: At the beginning of space flight, the investigations were oriented essentially toward the health of the astronauts. But in the last three decades space biology has evolved from 'try-and-see' experiments to sophisticated basic and applied research with well-based hypotheses as well as studies on the use of low gravity in biological applications. In 1977 the Space Biology Group of the Swiss Federal Institute of Technology, Zürich, began its activities in space research. A summary of the experiments performed to date, from basic research on the human immune system to the development of sophisticated instruments for biotechnology and medical application, is presented here.

Keywords: Bioreactor · Lymphocytes · Microgravity · Space Biology Group · Tissue engineering

1. Introduction

In 1977 the Space Biology Group of the Swiss Federal Institute of Technology, Zürich, began its activities in space research. And since January 1993 it has its premises in the ETH-wing of the Technopark. In addition to basic research and the development of biological instruments for space experimentation, our group is also involved in biotechnology, as well as in biocompatibility tests of materials, functionality tests and quality control of equipment for biotechnology and medicine from bread-board to flight units, consultancy to the Aerospace industry and studies on the preservation of biological materials. Three years ago we began to participate in medically oriented research on tissue engineering. And last but not least, the group has been chosen by ESA to become an USOC (User Support and Operations Center) for Biology and Biotechnology to support the scientists whose experiments will be on the International Space Station (ISS).

Space biology is a relatively young science that has evolved from the scientists' need to better understand the effects of the space environment on living systems. At the beginning of space flight, the investigations were oriented essentially toward the health of the astronauts, so that medical and physiological experiments predominated. Even if this aspect is still very important, today's investigators are increasingly interested in basic, material and applied research in microgravity.

The peculiarities of space environment are a reduced gravity (*i.e.* Shuttle: 10^{-2}–10^{-4} *g*), the quasi absence of motion by thermal or density driven convection and a high cosmic radiation (0.3 mSv/d on average for an astronaut inside the Shuttle). These physical changes create a new environment to which cells react in a more or less intensive way.

2. Basic Research

Experiments with cells from all steps of the evolutionary ladder have shown that important physiological functions in single cells can be dramatically altered at low gravity. Human white blood cells (lymphocytes), bone cells and epidermoid cells are among the cells most affected. Other cells like hybridoma, human embryonic kidney cells, and yeast cells are little or not at all affected by low gravity.

Russian scientists first reported the immune depression of astronauts in 1973. Since then, several laboratories, including ours, have been deeply involved in the study of gravitational effects on the cells of the immune system. Dr. Augusto Cogoli, the head of the Space Biology Group, has been investigating the effect of 0 *g* on T lymphocytes since his first experiment in 1983. These cells are one of the two main lymphocyte subpopulations and are responsible for the cellular immune response. The other lymphocyte subpopulation consists of B cells that are responsible for the synthesis and secretion of antibodies. *In vivo* the cells of the immune system are activated by the presence of foreign substances (antigens) such as bacteria, pollen or viruses. T lymphocytes may be activated *in vitro* by several substances, called mitogens, which are able to mimic the events occurring *in vivo* following exposure to an antigen.

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Our experiments have been based on three experimental approaches:

(i) *In vitro* experiments are carried out with cultures of immune cells purified from the blood of healthy donors (not necessarily astronauts);

(ii) *ex vivo* investigations are performed with cultures of blood samples drawn from the astronauts;

(iii) *in vivo* studies are based on the measurement of immunological parameters directly on the astronauts.

The goal of the *in vitro* investigation is to study the biological mechanism of T cells activation under the influence of gravitational changes. The objectives of the two other types of experiment are to assess the efficiency of the immune system in humans exposed to the stress of space flight. A comparison of these lines of investigation has helped to distinguish between effects of gravity and effects of physical and psychological stress. Our research was conducted in orbit (Biokosmos, MIR, Spacelab, Spacelab), in sounding rockets (MASER, MAXUS) as well as on the ground in the centrifuge for the hypergravity and in the clinostat for the simulated microgravity experiments. The Table gives a summary of the list of the experiments in which we have participated.

2.1. *In vitro*

Our laboratory was among the first to report on changes of the behavior of lymphocytes under altered gravitational conditions. These cells were 20–50% more activated when cultured at 10 *g* in a centrifuge. In 1980, we detected that under simulated hypogravity (0.02 *g*) in a fast rotating clinostat, the activation of lymphocytes was inhibited by 50%. An *in vitro* activation of lymphocytes in space was carried out for the first time in 1983 on the STS-9 Spacelab 1 mission. The activation was depressed by nearly 90% in microgravity. Experiments on stratospheric balloons demonstrated that the activation was not influenced by the cosmic radiation and therefore the depression was solely a consequence of the exposure of the cells to microgravity. This surprising discovery became the starting point of a series of experiments aimed to clarify certain aspects of the biological mechanism of T cells activation, such as aggregation, cell motility, and cytoskeletal structure. The results obtained on sounding rocket and in shuttle missions showed that cells are able to move and to aggregate under space conditions, but revealed that the micro-filaments of vimentin, a protein of the cytoskeleton, are altered in their structure after only 30 s exposure to microgravity (Fig. 1).

Table. List of the experiments conducted by the Space Biology Group of the ETH-Zürich

Mission name	Year	Experiment
STS-8	1983	Test flight of the incubator with human embryonic kidney cells
STS-9, Spacelab 1	1983	Lymphocyte activation
STS-61-A, D1	1985	2 experiments: Lymphocyte cultures, Lymphocytes from astronauts
STS-40, Spacelab SLS-1	1991	2 experiments: Lymphocyte cultures, Lymphocytes from astronauts
STS-42, Spacelab IML-1	1992	Hybridoma cells, DCCS with Hamster embryonic kidney cells, Friend cells
STS-65, Spacelab IML-2	1994	Lymphocyte activation and motion, Bioreactor
STS-76, 3rd Shuttle to MIR	1996	Bioreactor
STS-81, 5th Shuttle to MIR	1997	Preservation
STS-107 –Shuttle accident	2003	Bioreactor (stress reaction of yeasts); Lymphocytes (genetic expression)
MIR Mission 7	1988	'Skin-test' applicator
MIR Mission 8	1989	'Skin-test' applicator
MIR Mission 9	1990	'Skin-test' applicator
Biosatellite 9	1989	Test of the DCCS with protoplasts
MASER 3	1989	Lymphocytes: mitogen binding, patching and capping
MASER 4	1990	Lymphocytes: cytoskeleton, mitogen binding, patching and capping
MASER 9	2002	Chondrocytes genetic expression, Hardware CODI testing
MAXUS 1 –Rocket failure	1991	Lymphocytes: motility, cytoskeleton, mitogen binding, patching and capping
MAXUS 1b	1992	Lymphocytes: cytoskeleton, mitogen binding, patching and capping
MAXUS 2	1995	Lymphocytes: motility, cytoskeleton, mitogen binding, patching and capping
Stratospheric balloon, program ODISSEA –Balloon failure	1986	Cosmic radiation and lymphocyte activation
Stratospheric balloon, program ODISSEA	1987	Cosmic radiation and lymphocyte activation

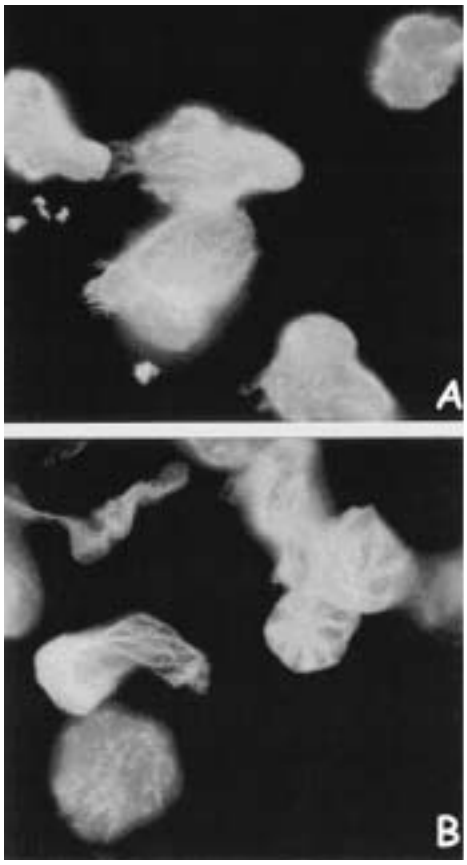

Fig. 1. Alteration of the cytoskeleton structure: cytofluorographs of the intermediate filaments of vimentin of Jurkat cells. A: Ground control; B: Flight samples after 30 s in microgravity.

2.2. *Ex vivo*

Our laboratory was the first and to date the only one to conduct mitogenic activation with astronauts' blood during flight. The results obtained in different flights were contradictory, but the documentation gathered by the American as well as by the Russian investigators on a total of 129 subjects showed a depression of the activation of the lymphocytes in 56% of the tested crew members. As in our experiment, recovery occurred within two weeks after landing and no health consequences have been reported.

2.3. *In vivo*

In vivo experiments – application on the skin of standardized and sterile antigen and toxin preparations – gave the same variation in the response as for the *ex vivo* experiments. But a reduction of the reaction was often correlated with significant stress. Such a reaction was also observed for persons involved in long-term physical exercise.

The data reviewed here clearly indicate that important effects are detected in the cells of the immune response exposed *in vivo* as well as *in vitro* to the space environment. However, the correlation between the depression seen in free-floating cells (*in vitro*) and that measured in the cells of the astronauts during and after flight (*ex vivo*) is only apparent. The impact of space flight on the immune response of astronauts is evident. *In vivo*, the alteration of the immune response is associated with factors of the space flight such as stress, radiation, confinement, changes in the circadian rhythm and microgravity. More work is certainly necessary to establish the risks of infection related to long-duration flights and to design countermeasures.

3. Instrument Development

From the beginning we were confronted with the need to have instruments not only 'space qualified' but also specifically adapted to the requirements of our experiments. Such apparatus was not commercially available; therefore we have begun to actively participate in the development of our equipment. Experiment-specific hardware not only has to meet the requirements of the experimenters specifically, but it also needs to cope with the strict place, power and safety constraints of the space agencies.

For our first experiment in 1983, we needed an incubator with culture chambers capable of maintaining a constant temperature of 37 °C (±1 °C), and which could be fixed to a front panel installed in the Space Shuttle's flight deck or in a rack of the Spacelab module. The traditionally used plastic culture flasks are not suitable for working in space (too fragile and flammable), therefore new culture chambers had to be developed. The first step to overcome was to select biocompatible materials. Another problem was raised by the necessity to develop a chamber into which it should be possible to inject liquid without having any air inside and without leakage. The solution of the problem was a culture chamber hermetically sealed with a mobile piston. Before being accepted for flight by the space agencies, the hardware must also go through space qualification tests such as vibration, off-gassing and electromagnetic contamination tests. The principle of the mobile piston was adopted later for culture chambers.

All the early experiments performed in space were so-called batch experiments. A batch experiment is an experiment in which the cells are cultivated in a fixed amount of nutrient solution, limiting the time of cultivation. To remedy to this problem we developed, in collaboration with Contraves AG, a totally automatic cultivation instrument for animal cells with continuous delivery of fresh medium. The advantage of such cultivation is that the cells can grow for several days without being starved. The PRODEX program of ESA funded this development.

This program funds the development of specific space hardware accepted for flight in the member countries of the ESA that do not have their own space agency such as Switzerland, Austria or Belgium. This first system was called Dynamic Cell Culture System. The novelty of this system was the self-powered osmotic pump supplying the cells with fresh medium at a continuous flow rate of 1 µl h^{-1}. Its biological performance was tested on the Biokosmos 9 satellite (1989) with plant protoplasts. Aboard the IML-1 mission (STS-42, 1992) the DCCS was used for the cultivation of hamster kidney cells. Our next goal was to overcome the limitations of the DCCS (small volume of culture) and to develop a miniaturized bioreactor with sampling capability, pH control, gas exchange, continuous fresh medium supply, and on-line measurements. The real challenge of this development was the size of the container in which it should fit (87×63×63 mm, Fig. 2). This bioreactor was built in collaboration with Mecanex SA and the Institute of Microtechnology of the University of Neuchâtel with PRODEX funding. It is designed for yeast cell cultivation. This bioreactor flew aboard the Shuttle missions STS-65 and STS-76 (1994, 1996). A second gen-

Fig. 2. Miniaturized bioreactor with two syringes for the sampling and the sample bottles. The cultivation chamber is on the top. The inspection window is located on the right upper side. The bottom structure contains the fresh and the used medium. Total height 8 cm.

eration of space bioreactor with a larger working volume had flown on mission STS-107; due to the tragic accident of Columbia the data were lost.

4. Tissue Engineering

Three years ago our group has became involved in collaboration with several European scientists and Centerpulse (former Sulzer Medica), in a tissue-engineering project selected by ESA within the application and commercialization program of the International Space Station. The first objectives of the project were: 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. In a second phase the project focuses on the growth of cartilage.

Our group is also involved in studies on follicular thyroid carcinoma cells, on osteoclasts, and on cardiovascular tissue.

5. BIOTESC

As part of the international ground segment under development for the International Space Station (ISS), and according to the European Decentralization Operations Scheme, a Facility Support Center (FSC) for Biolab has been established in Zürich under the supervision of the ETH Space Biology Group, in support of DLR/MUSC in Cologne, which acts as the Biolab Facility Responsible Center (FRC). The center is referred to as ETH/Biotesc-Biotechnology Space Support Center. Biolab offers a research tool for biological studies on cell cultures, micro-organisms, small plants and small invertebrates. This new ESA center was officially inaugurated at the end of June, 2000. At present various documents defining the frame and the activities of the USOCs are under development in what is called the Definition Phase. The next phase will be the Implementation Phase, planned to start soon.

6. Conclusions

The achievements of our activities described in the previous sections have contributed substantially to the development of three directions in space biology and medicine. First, the use of microgravity to investigate the mechanism of activation on T lymphocytes permitted the study of a complex and still obscure aspect of biology. Second, important new knowledge was gained on the effect of stress on the human immune system. Third, the development of sophisticated instruments and of technologies to handle biological specimen in weightlessness paved the way to future endeavors in biotechnology on board the International Space Station. Large efforts are presently undertaken to design the new facilities for that new space laboratory.

There is also another motivation to continue our and our colleagues' work in space biology in the future even though space flights are still very risky. Human exploration of space is just at its beginning. Beyond the International Space Station human settlements will be established on the Moon and on Mars in the next decades. Later new space vehicles will carry humans on longer interplanetary travels to other planets and to their satellites. Therefore we consider such activities also as a contribution to the understanding of the behavior in and of the adaptation of terrestrial life (from simple unicellular organisms to humans) to the space environment.

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