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Effect of Microgravity on Human T Lymphocyte Activation: Experiments in Spacelab and Sounding Rockets

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Several physiological systems are altered by the environment of spaceflight. The results of an experiment carried out in Spacelab-1 have surprisingly shown that the mitogenic activation with Concanavalin A (Con A) of T lymphocytes is nearly completely inhibited (by more than 95%) in microgravity conditions [1]. We have investigated the causes of the phenomenon in a series of studies performed in the last 15 years in 6 Spacelab missions (D-1 in 1985, SLS-1 in 1991, IML-1 in 1992, IML-2 in 1994 and dramatic STS-107 in 2003), in sounding rockets (6 missions) and in ground-based simulations in the clinostat. Immune cells have been chosen by us and several other research groups as an important model to study *in vitro* the influence of microgravity on cell differentiation and genetic expression following transduction of an external signal, on cell motility, formation of cell aggregates and cytoskeleton. The experiments have been carried out with peripheral blood lymphocytes and monocytes, with purified T lymphocytes or with Jurkat cells. The firsts to study cultures of lymphocytes in space were Tálas et al. [2] who discovered that the production of interferon-alpha (IFN-a) induced by polynucleotides in human lymphocytes cultured on the soviet spaceship Salyut 7 was increased by 500% compared to the ground controls. Important effects were observed by Chapes et al. [3] in cultures of three types of immune cells in space. The anchorage-dependent bone marrow-derived macrophage cell line B6MP102 secreted, upon activation with lipopolysaccharide, significantly more interleukin 1 (IL-1) and IFN-g in space than on the ground. Murine spleen cells stimulated with poly I:C released significantly more IFN-a in space than on Earth. Also, human PBL as well as murine lymph node T cells activated with Con A produced significantly more IFN-g in space than on Earth. Another interesting investigation was carried out on the Russian biosatellite Cosmos 2044 with THP-1 monocytes and Jurkat cells, phenotypically similar to T cells, by Limouse et al. [4]. Although normal production of interleukin 2 (IL-2) and IL-1 was observed in Jurkat cells activated with monoclonal antibodies directed against CD3/T in the presence of THP-1 cells, the production of IL-1 and IL-2 was dramatically inhibited when the cells were individually activated with phorbol ester (an activator of protein kinase C) and calcium ionophore A 23187, thus indicating that the function of protein kinase C (PKC) may be directly affected by microgravity. In an experiment conducted with human leukocytes in IML-2 Schmitt et al. [5] showed that the distribution of PKC, a key element of the signal transduction of T cells is altered in microgravity. Recently, Cooper and Pellis [6] reported that the loss of activation of T cells in microgravity, simulated in a device called rotating wall vessel, was restored by direct activation of PKC with phorbol myristate. However, such restoration was only partial under optimum activation conditions. The outcome of all these investigations clearly demonstrates that the behaviour of immune cells in cultures - lymphocytes as well as monocytes - is influenced by gravity changes.

Most of the work performed by our group is related to the study of different aspects of the mitogenic *in vitro* activation of human lymphocytes under altered gravitational conditions. The mechanism of the mitogenic activation is very complex. At least three different signals are required: the first is the binding of the mitogen Con A to the cell membrane; the second is delivered by IL-1 produced by monocytes always present in lymphocyte cultures and the third is IL-2 produced by the T cells itself. Cell-cell interaction is an important element of signal transduction in immune cells. In particular, the transmission of the second activation signal in T lymphocytes is probably linked to direct contact with monocytes .

1st activation signal. Experiments in sounding rockets (providing microgravity periods of 6-12 min) showed that the binding of Con A occurs regularly at low g, whereas patching of the Con A ligands is significantly retarded [7]. Data from an experiment on the sounding rocket MAXUS 1b show that important changes in the microfilament structure of vimentin and tubulin occur 30 s after exposure to microgravity [8]. Most evident is the appearance of large bundles in a significant higher percentage of cells compared to the ground control. The other statistically significant alterations observed in microgravity consist of the formation of aggregates of protein, suggesting depolimerization processes, as well as of discontinuities of the filamentous network. The results have been confirmed with an experiment performed on MAXUS 2: an in-flight 1 *g* centrifuge allowed the comparison of the data obtained in microgravity with a 1 *g* control having the same history related to launch and re-entry. These investigations have been performed with Jurkat cells, a cell line derived from a human T cell leukemia. The higher cytoplasmic volume and size of these cells, compared to resting lymphocytes, render them more suitable for this type of analysis [7].

2nd activation signal. Data from studies in the sounding rocket Maxus 1b [8] and in Spacelab [9] showed that human T

lymphocytes are capable of autonomous movements, formation of aggregates and, therefore, of cell-cell interactions at low g. This is probably a pre-requisite for the transduction of the second activation signal. Contradictory data concerning the production of the 2nd signal, IL-1, by monocytes were obtained in two Spacelab experiments. In Spacelab SLS-1 the secretion of IL-1 was strongly depressed at 0xg [10]. Based on such data, exogenous IL-1 was added to the cultures in a following experiment in Spacelab IML-2. While the loss of activation could not be restored, the endogenous production of IL-1 was enhanced [11].

3d activation signal. While the secretion of IL-2 is only slightly depressed at 0xg and thus there is a sufficient amount to trigger the 3rd activation phase of T cells, the amount of IL-2R found in the supernatant of cultures at 0xg is strongly depressed [11]. Although the evidence is indirect, it is speculated that a failure of the expression of IL-2R (in particular of the IL-2Ra subunit) is the cause of depressed activation at low g. Indeed, an investigation in moduled microgravity conditions, using a threedimensional clinostat (Random Positioning Machine), revealed a reduced expression of IL-2Ra mRNA, but not of IL-2Rb [12]. Recently we used cDNA microarray hybridization technology to monitor the transcriptional response of activated human T lymphocytes to 6 minutes of microgravity during the MASER 9 flight. By comparing flight samples with ground controls and with flight centrifuge controls, we found that in purified T lymphocytes stimulated with Con A and anti-CD28 immediately after the onset of microgravity, about 1% of the 4.400 genes monitored showed significant modulations in response to low g, including down regulation of IL-2Ra [13]. The genetic expression of cytokines and of IL-2 receptor subunits was the objective of our unlackely experiment in real microgravity lost on the catastrofic space Columbia shuttle mission STS-107 in January 2003.

Apoptotic effects in lymphocytes cultured at low g have been observed recently by our group [14] and by Lewis et al [15]. Since in space living organisms, including cells, are affected by microgravity and cosmic radiations, we are also investigating the effects of cosmic radiations on the genetic expression in human T lymphocytes, using stratospheric balloon flights.

Key words

microgravity, lymphocytes, cytokines

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