

**Signal Transduction in T Lymphocytes in Microgravity**

Augusto Cogoli  
*Space Biology, ETH Technopark, Technoparkstrasse 1 Ch-8005 Zurich, Switzerland*

**ABSTRACT**

More than 120 experiments conducted in space in the last 15 years have shown that dramatic changes are occurring in several types of single cells during their exposure to microgravity. One focus of today's research on cells in space is on signal transduction, especially those steps involving the cytoskeleton and cell-cell interactions. Signal transduction is often altered in microgravity as well as in hypergravity. This leads to changes in cell proliferation, genetic expression and differentiation. Interesting examples are leukocytes, HeLa cells, epidermoid cells and osteoblastic cells. Signalling pathways were studied in T lymphocytes in microgravity by several investigators after the discovery that mitogenic activation *in vitro* is virtually nil at 0g. T cells are a good model to study signal transduction because three extracellular signals (mitogen, IL-1 and IL-2) are required for full activation, and two classical pathways (via proteins G and PKC) are activated within the cell. In addition, low molecular weight GTP-binding proteins (Ras and Rap) are interacting with the cytoskeleton. The data at 0g support the notion that the expression of IL-2 receptor is inhibited at 0g, while mitogen binding and the transmission of IL-1 by accessory cells occurs normally. In addition, alterations of the cytoskeleton suggest that the interaction with Rap proteins is disturbed. Data obtained with phorbol esters indicate that the function of PKC is changed in microgravity. Similar conclusions are drawn from the results with epidermoid cells A431.

**INTRODUCTION**

The knowledge of signal transduction mechanisms and pathways in the cell has made important progress recently. In particular, T lymphocyte activation is one of the most studied subjects. There are two main reasons: first, it is an interesting and intriguing biological process, second, it is one of the key events regulating the immune response. Its failure (as in case of HIV infections) leads to irreparable damages.

Remarkable findings were made in the last decade also in space and gravitational cell biology. Experiments conducted by European, Japanese and US investigators in dedicated space missions (Spacelab D-1 in 1985, SLS-1 in 1991, IML-1 in 1992, IML-2 in 1994 and in several sounding rockets flights between 1985-95) as well as in simulations on Earth have shown that single cells are sensitive to changes of the gravitational environment. Several types of mammalian cells showed important changes in proliferation, differentiation and genetic expression. The cells of the immune system, T lymphocytes and monocytes in particular, are severely affected by the exposure to 0g. However, most of the experiments conducted so far were dedicated to the search for rather unpredictable effects (so called "fishing experiments") based on vague hypotheses and assuming that alterations of the gravitational environment are likely to alter the behaviour of the cell. Once important effects were identified, a second phase began that is dedicated to the understanding of the phenomena on a molecular basis. This second phase is now in progress and it is based on the application of the most sophisticated and new technologies of molecular biology. However, such procedures are still too complicated to be carried out in space laboratories due to safety constraints and lack of adequate facilities. Most analyses are conducted on the ground after fixation and preservation of the biological probes in space. The purpose of this article is to describe the gravitational effects discovered in T lymphocytes and to compare the data with what is known on their activation mechanism.

**SIGNAL TRANSDUCTION PATHWAYS**

The series of events leading to full T lymphocyte activation is a very complex process, several aspects of which are still unclear and controversial. Some of such steps are common to several types of cells (Rasmussen et al, 1992, Rasmussen, 1995, Berridge, 1993), some are peculiar to T cells. The response of T cells is manifold and consists essentially of two types: First, after expression of early genes, a number of cytokines is secreted. Among them, interferon- and interleukin-2 (IL-2). Second, the cells which are in a "resting" G0 phase are induced to enter the cell cycle ending with the mitosis. This process takes approximately 70 hours and it is characterised by important morphological changes. The expression of IL-2 and of its receptor is a key element of the activation (Gaulton and Williamson, 1994).

The activation mechanism is the object of extended investigations and the description of each single step may vary considerably depending on the interpretation of different authors. An old but useful and clear overview of the general pathway is that proposed by Berridge (1985), whereas a description specific for T lymphocytes was proposed by Palacios (1982). A mechanism consisting of three phases and consistent with several data of the literature (reviewed by Cogoli, 1993) is presented in Figure 1. Concomitant with the three phases, three signals are almost certainly required for full T cell activation.

**Phase A.** Concanavalin A (Con A), a lectin carrying four binding sites specific for alpha-glucosides, binds to membrane glycoproteins (MGP) with consequent
Fig. 1. Three-signal model of mitogenic signal transduction in T lymphocytes. (legend continued next page)
Top left: outline of the three signals. Below left: mechanism of activation (from Cogoli, 1993). Three phases of T cell activation can be distinguished. **Phase A:** Binding of Con A to membrane glycoproteins (MGP), induction of protein G (G) to activate phospholipase C (PLC). Cleavage of phosphoinositol-bisphosphate (PIP₂) into diacylglycerol (DG) and inositol trisphosphate (IP₃). Release of calcium ions from the endoplasmic reticulum (ER). Activation of a cytoplasmic kinase. Triggering of the synthesis of IL-2 after the expression of the oncogenes c-fos and c-myc. **Phase B:** Secretion of IL-2 and cell-cell interaction between T and accessory cells (AC, usually monocytes), triggering of the production and secretion of IL-1, activation of protein kinase C (PKC) upon interaction with IL-1. Probably synergistic effect of DG, activation of a cytoplasmic protein. Production and insertion of IL-2R in the cell membrane. Amplification of synthesis and secretion of IL-2. **Phase C:** Upon interaction between IL-2 and its receptor, the full activation of T lymphocytes is triggered. Cells start to divide and two populations, the effector and the memory cells, are generated. Right: IL-2R coupled signalling cascade (from Gaulton & Williamson, 1994).

The association mode of the three subunits regulates the affinity for IL-2 and the signalling function of the IL-2/IL-2R complex. The signalling cascade following activation of the IL-2R is presented in Figure 1.

The use of specific inhibitors has contributed recently to the clarification of certain details of the signalling events. The inhibition of mitogen-induced T cell proliferation by pentoxifylline is associated with a marked reduction of IL-2R (called also CD25) expression (Rieckmann et al., 1996). On the other hand, rapamycin inhibits CD25 upregulation but not IL-2 synthesis (Wooery et al., 1996). Specific inhibition of protein-tyrosine kinase inhibits the induction of IL-2 gene but not that of IL-2R (Hanke et al., 1996). Concerning the time-sequence of events, IL-2 is expressed 45 min after activation, whereas IL-2R is expressed after 2 h (reviewed by Crabtree, 1989). In the cell subsets CD4 + CD45R the expression of IL-2R occurs within 4 h of activation (Beckman et al., 1994). Although glutamine is required for T stimulation, mRNA coding for IL-2 and IL-2R was detected independently from exogenous glutamine supply (Horig et al., 1993). Distinct roles are played by the T cell receptor which regulates the function of phosphatidylinositol 3-kinase (phase A of activation) and by the IL-2R which regulates p21Ras (Cantrell et al., 1993). Phosphatidic acid is essential for T cell proliferation, and its generation is induced by IL-2 (Flores et al., 1996). IL-2 mRNA and IL-2R mRNA, respectively, show different degradation and stability (Bill et al., 1994). Tumor necrosis factor- (TNF-) plays an important role in the expression of IL-2R (Pimentel-Muinos et al., 1994).

**GRAVITATIONAL EFFECTS ON SIGNAL TRANSDUCTION**

A summary of the data on signal transduction is given in Table I. Reviews on this topic appeared recently (Claassen and Spooner, 1994; Moore and Cogoli, 1996). The data of a series of biological experiments performed in the Spacelab flight IML-2 in 1994 were published in a special issue of the Journal of Biotechnology edited by the author (Cogoli, 1996). The data available today clearly document that single cells may be sensitive to alterations of the gravitational environment.
Table 1. Gravitational effects on signal transduction, genetic expression and metabolism in mammalian cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Effect</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypergravity in Centrifuges</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HeLa cells, human</td>
<td>Elevated levels of c-myc mRNA at 18xg, 35xg and 70xg; effect most evident at 35xg; levels of c-myc 3-3.8 times higher than control. Increase of inositol 1,4,5-triphosphate at 35xg: 1.5 times after 2 minutes; 2.1 times after 5 minutes. Decrease of cyclic adenosine monophosphate at 35xg: 11% after 10 minutes and 16% after 20 minutes.</td>
<td></td>
<td>Kumei et al, 1989.</td>
</tr>
<tr>
<td>A431 epidermoid cells, human</td>
<td>18% increase of growth factor induced c-fos expression at 10xg; no change of constitutive expression.</td>
<td></td>
<td>de Groot et al, 1991a, 1991b.</td>
</tr>
<tr>
<td><strong>Hypogravity in Clinostats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-562 cells, human</td>
<td>Exposed to hemin: 10% decrease in glucose consumption, 50% decrease in hemoglobin production; unchanged number of hemoglobin-producing cells.</td>
<td>Hemin induces the expression of hemoglobin.</td>
<td>Wiese et al, 1988.</td>
</tr>
<tr>
<td>A431 epidermoid cells, human</td>
<td>20-25% depression of epidermal growth factor-induced c-fos expression; no change in constitutive c-fos mRNA level; c-fos expression induced by phorbol ester reduced by 30%, no effect on A23187 or forskolin induced c-fos expression.</td>
<td>Phorbol esters activate PKC, forskolin activate PKA, A23187 and phorbol esters mimic the effect of epidermal growth factor.</td>
<td>deGroot et al, 1990, 1991a, 1991b.</td>
</tr>
<tr>
<td><strong>Microgravity in Space</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T lymphocytes with monocytes as accessory cells, human</td>
<td>500% increase of interferon-α secretion induced by various agents; Con A activation of cells attached to microcarrier beads: 2.5 fold increase in interferon-γ production and 2 fold increase in production of IL-2.</td>
<td>Salyut 6, incubator switched off during crew-sleep period; Spacelab in flight 1 g control.</td>
<td>Tálás et al, 1983. Bechler et al, 1992, Cogoli et al, 1993.</td>
</tr>
<tr>
<td>Monocytes as accessory cells in T lymphocyte culture, human</td>
<td>Contradictory results: Nearly total inhibition of IL-1 production in resuspended cells; normal IL-2 secretion.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jurkat cells, human T cell line</td>
<td>Normal production of IL-2 after induction with anti-CD3 monoclonal antibodies in the presence of TIP-1 cells; 100% inhibition of IL-2 production induced by calcium ionophor and phorbol ester. The distribution of PKC is altered in microgravity</td>
<td>Russian biosatellite.</td>
<td>Limouse et al, 1991.</td>
</tr>
<tr>
<td>THP-1, myelomonocytic cell line</td>
<td>Normal production of IL-1β after induction with anti-CD3 monoclonal antibodies in the presence of Jurkat cells; 85% inhibition of IL-1β production induced by phorbol ester.</td>
<td>Russian biosatellite.</td>
<td>Limouse et al, 1991.</td>
</tr>
<tr>
<td>7E3 hybridoma cells</td>
<td>Production of monoclonal antibodies, consumption of glucose and glutamin as well as secretion of lactate and ammonia decreased.</td>
<td></td>
<td>Bechler et al, 1993.</td>
</tr>
<tr>
<td>Cell type</td>
<td>Effect</td>
<td>Remarks</td>
<td>Ref.</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>B6MP102 macrophage cell line</td>
<td>Increased secretion of IL-1 and interferon-γ induced by lipopolysaccharide.</td>
<td>Space Shuttle mid-deck, ambient temperature, no 1g control.</td>
<td>Chapes et al., 1992.</td>
</tr>
<tr>
<td>Bone-marrow-derived macrophages, mice femora and tibiae</td>
<td>150% increase of IL-6 secretion, up to 100% decrease of phenotypic marker expression of MHC-II and MAC-2.</td>
<td>Space shuttle STS-57, 60, 62; no 1g control, incubation between 23 and 27°C.</td>
<td>Armstrong et al., 1995.</td>
</tr>
<tr>
<td>Friend leukemia-virus transformed cells, murine</td>
<td>No changes in metabolic behaviour: glucose and glutamin consumption, production of lactate and ammonia in dimethylsuloxide induced production of hemoglobin.</td>
<td>Spacelab, in flight 1g control.</td>
<td>Bechler et al., 1993.</td>
</tr>
<tr>
<td>A 431 epidermoid cells, human</td>
<td>50% depression of epidermal growth factor induced expression of c-fos and c-jun; induction with phorbolester: 26% depression of c-fos and 51% depression of c-jun expression.</td>
<td>2 experiments in sounding rockets.</td>
<td>deGroot et al., 1990; 1991b.</td>
</tr>
<tr>
<td>Kidney cells, hamster</td>
<td>No effect on metabolism and production of tissue plasminogen activator.</td>
<td>Spacelab IML-1, 1g control.</td>
<td>Lorenzi et al., 1993.</td>
</tr>
<tr>
<td>L929 cells from connective tissue, murine</td>
<td>Inhibition of TNF-α mediated killing; inhibitors of PKC restored TNF-α mediated cytotoxicity in dose-dependent manner.</td>
<td>Cultures at ambient temperature in Shuttle mid-deck, no 1g control; cultures at „approximately“ 37°C in Shuttle middeck, no 1g control.</td>
<td>Chapes et al., 1994; Woods et al., 1994.</td>
</tr>
<tr>
<td>Osteoblasts, rat</td>
<td>4.5-136-fold increase of prostaglandin E2 production, 3.3-9.5-fold increase of prostaglandin G/H synthase mRNA, 6.4-9.3-fold increase of IL-6 production.</td>
<td>Spacelab, no 1g control in flight</td>
<td>Kumei et al., 1996.</td>
</tr>
<tr>
<td>Pituitary cells, rat</td>
<td>Complex microgravity-related interaction between frequency of cell feeding and quantity/quality (biological activity) of 6 hormones.</td>
<td>Spacelab, no 1g control in flight</td>
<td>Hymer et al., 1996.</td>
</tr>
</tbody>
</table>
A near total inhibition of the activation of T cells by Con A, determined as the mitotic index, was discovered in 1983 in an experiment in Spacelab 1 (Cogoli et al., 1984). Preliminary studies conducted in the fast rotating clinostat in the early eighties showed an inhibition of approximately 50% (Cogoli et al., 1980). Such results were confirmed later in several experiments in space (Bechler et al., 1986, 1992, Cogoli et al., 1993, Pippia et al., 1996). A strategy based on the study of the transduction of the three signals led to a series of interesting results:

1st activation signal. Experiments in sounding rocket flights (providing microgravity periods of 7-12 min) showed that the binding of Con A occurs regularly at 0g, whereas patching and capping of the Con A ligands are slightly retarded (Cogoli M. et al., 1992, Cogoli-Greuter et al., 1994). Fluorescent labelled Con A was injected at pre-programmed times into cultures of leukocytes by means of an automatic device followed by in-flight fixation with paraformaldehyde.

2nd activation signal. Data from studies in the sounding rocket Maxus 1 and 2 (Cogoli-Greuter et al., 1994) and in Spacelab IML-2 (Cogoli-Greuter et al., 1996) showed that leukocytes are capable of autonomous
movements, formation of aggregates and, therefore, of cell-cell interactions at 0g. This is probably a pre-requisite for the transduction of the second activation signal. Interestingly enough, gravity cannot be the driving force of such movements in contrast to the cell motion described by Stossel (1993). In fact, the hydrostatic pressure (which is due to gravity) on "focal defects" of the cell membrane generates protrusions of the membrane with consequent "crawling" of the cells. Contradictory data concerning the production of the 2nd signal, IL-1, by monocytes were obtained in two Spacelab experiments. In Spacelab SLS-1 we saw that the secretion of IL-1 was strongly depressed at 0g (Bechler et al. 1992, Cogoli et al. 1993). Based on such data, exogenous IL-1 was added to the cultures in a following experiment in Spacelab IML-2. As shown in Figure 2, while the loss of activation could not be restored, the endogenous production of IL-1 was normal (Pippia et al. 1996).

3rd activation signal. While the secretion of IL-2 is only slightly depressed at 0g 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 0g is strongly depressed as shown in Figure 2 (Cogoli et al. 1993, Pippia et al. 1996). Although the evidence is indirect (we do not have data on the specific expression of IL-2R mRNA nor of its insertion in the membrane), we speculate that a failure of the expression of IL-2R is the cause of depressed activation at 0g.

Cytoskeleton. Data from two experiments in Maxus 1 and 2 show important changes of the microfilament structure (actin and vimentin) occur 30 s after exposure to microgravity (Cogoli-Greuter et al. 1994; unpublished data). The cytoskeleton plays an important role in the regulation of certain cellular functions. In particular, the small G-protein Rho seems to interact with the cytoskeleton (reviewed by Quinn, 1995). The role of Rho in the regulation of phosphatidylinositol 4-phosphate 5-kinase (Chong et al. 1994) and in lymphocyte-mediated cytotoxicity (Lang et al. 1992) has been described recently.

Anchored cells. Another unexpected result of an experiment in Spacelab SLS-1 is the two-fold increase of T cell activation in cultures of cells attached to microcarrier beads (Cogoli et al. 1993). We do not have yet an explanation nor an hypothesis to explain such finding.

Threshold of gravisensing. Unpublished data from an experiment on Spacelab SLS-1 revealed there is a sensitivity threshold between 0.6 and 0g in T lymphocytes exposed to Con A (Cogoli et al., unpublished).

Blood samples drawn in-flight from 4 crew members and diluted with culture medium were cultured in the presence of Con A at 37°C in a multi-g centrifuge at 0.6, 1.0, 1.3 and 1.7 g and in an incubator at 0g, respectively. As shown in Figure 3, activation was nearly nil at 0g in all samples, whereas it was close to the levels of the respective 1g controls in two cases (crew members A,B) and, although lower than that of the 1g controls, clearly above 0 in the other two cases (crew members C,D).

In 1989 Limouze et al. performed an experiment on the Soviet biosatellite Cosmos 2044 with THP-1 monocytes and Jurkat cells, which are phenotypically similar to T-lymphocytes (Limouze et al. 1991). While normal production of IL-2 and IL-1 was observed in a system consisting of Jurkat cells activated with monoclonal antibodies directed against CD3/ T in the presence of THP-1 cells, the production of IL-1 (by THP-1 cells) and of IL-2 (by Jurkat cells) was dramatically inhibited when the cells were individually activated with PMA - an activator of protein kinase C - and calcium ionophore A 23187 thus indicating that the function of protein kinase C may be directly affected by gravity. This assumption is also supported by the results of De Groot et al. who reported that the expression of c-fos and c-jun proto-oncogenes induced by epidermal growth factor and phorbol ester in human A431 epidermoid carcinoma cells was markedly decreased in microgravity (deGroot et al., 1991a,b). Conversely, the expression of c-fos and c-jun induced either by forskolin or A 23187 - both known to induce protein kinase C - is not affected. In an experiment conducted with human leukocytes in IML-2, Schmitt et al. (1996) showed that the distribution of PKC is altered in microgravity.

Important effects were observed by Chapes et al. in cultures of three types of immune cells in space (Chapes et al., 1992) and by Armstrong et al. in macrophages in parabolic flights (Armstrong et al., 1995). The anchorage-dependent bone marrow-derived macrophage cell line B6MP102 secreted, upon activation with lipopolysaccharide, significantly more IL-1 and TNF- in space than on ground. Murine spleen cells stimulated with poly I:C released significantly more interferon- in space than on earth. Also, free human peripheral blood lymphocytes as well as murine lymph node T cells activated with Con A produced significantly more interferon- in space than on earth. A strong increase of interferon- secretion by human lymphocytes was also found by Hungarian and Russian investigators on the space station Salyut-7 (Talas et al., 1983). Other data relevant to this topic are those published on the effects on signal transduction in HeLa cells (Kumei et al., 1989) and in osteoblasts (Kumei et al., 1996).

Investigations with other cell systems, carried out in space by our team, showed much less effects of microgravity: Hybridoma cells and cells transformed by Friend leukaemia virus (Bechler et al., 1993) as well as hamster kidney cells (Lorenzi et al., 1993) showed little or no changes in proliferation, metabolism or morphology.

In two investigations in Spacelab with Saccharomyces cerevisiae grown in a bioreactor developed in our laboratory in collaboration with Swiss aerospace industry, we detected an unusual distribution of the budding scars in cells grown at 0g (Walther et al., 1996). This points out again that important changes of the cytoskeleton may occur at 0g.
Fig. 3. Activation of T lymphocytes at variable-g in space. Blood samples were drawn in-flight from four crew members during the Spacelab SLS-1 mission. The blood was diluted 1:10 with culture medium and exposed to Con A for 3 days in cultures kept in a variable-g centrifuge at four g-levels (1.7g, 1.3g, 1.0g and 0.6g) and at 0g in an incubator, respectively. Activation was determined as the amount of radioactive thymidine incorporated into DNA. SEM values of triplicates are given.

CONCLUSIONS

Table II gives a synopsis of what is known today on the behaviour of T lymphocytes under altered gravitational conditions and on the hypotheses formulated to explain the phenomena observed. Extensive work is in progress in space (in particular in two flights of the Biorack facility in 1997 and on ground. In our laboratory a new instrument, the "centrifuge free-fall machine" invented by D. Mesland (Mesland, 1996; Mesland et al., 1996) is presently being tested with cultures of lymphocytes. Other investigations will begin soon with the three-dimensional clinostat (Hoson et al., 1992). It can be expected that new and surprising results will be obtained soon in this line of studies.

Acknowledgements

The work performed in the author’s laboratory has been supported by the Swiss National Science Foundation, SNSF, by the Board of the Swiss Federal Institute of Technology, ETHZ, by the PRODEX Programme of ESA, by Contraves AG, by Biostrath AG, by ESA, and by NASA. The support of the Biorack Team of ESA, of the Life Science Division of the NASA Johnson Space Center in Houston, of Bionetics of the Life Science Facilities at the NASA Kennedy Space Center is also gratefully acknowledged.

REFERENCES


<table>
<thead>
<tr>
<th>Activation phase</th>
<th>Question</th>
<th>Answer(^a)</th>
<th>Experimental approach</th>
<th>Hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> Extra-cellular</td>
<td>Binding of Con A to the cell membrane; Patching of the Con A ligands; Capping of the Con A ligands.</td>
<td>Yes; Yes, but retarded; Yes, but retarded</td>
<td>Fluorescent labelled Con A in sounding rockets MASER 4, and 5 (Cogoli M et al., 1992).</td>
<td>Alteration of the cytoskeleton affects the aggregation of Con A ligands.</td>
</tr>
<tr>
<td></td>
<td>Alteration of the cytoskeleton; Activation of G-proteins and PLC(^c); Early expression of proto-oncogenes.</td>
<td>Yes, significant changes after 30 s at 0kg; TBD; TBD(^b).</td>
<td>Immunofluorescence (actin, vimentin) in rocket MAXUS 1 (Cogoli M et al., 1994); Phosphorylation of G-proteins and PKC; RT-PCR.</td>
<td>Alteration of the interaction of G-proteins (rho) with the cytoskeleton may disturb the transduction of the 1st activation signal.</td>
</tr>
<tr>
<td><strong>2</strong> Extra-cellular</td>
<td>Cell-cell interaction/aggregation; Cell motion.</td>
<td>Yes, but less than at 1xg; Yes, as at 1xg.</td>
<td>Microscopy in sounding rocket MAXUS 1, NIZEMI microscope in Spacelab IML-2 (Cogoli M et al., 1994, Cogoli-Greuter et al., 1996).</td>
<td>The distribution of cells resuspended at 0kg may reduce the contacts compared to cells sedimented to the bottom of the culture vessel. However, the activation of monocytes to produce IL-1 is not affected.</td>
</tr>
<tr>
<td>Intra-cellular</td>
<td>Early secretion of II-2; Expression of II-1 by monocytes;</td>
<td>Yes, TBC; TBD, controversial results: inhibited in Spacelab SLS-1, unchanged in Spacelab IML-2;</td>
<td>Monoclonal antibodies in Spacelab SLS-1 and IML-2 (Cogoli et al., 1993, Pippia et al., 1996);</td>
<td>The function of accessory cells (monocytes) may be inhibited at 0kg;</td>
</tr>
<tr>
<td><strong>3</strong> Intra-cellular</td>
<td>Late secretion of II-2; Secretion of II-2R in the supernatant; Expression of II-2R; Expression of proto-oncogenes.</td>
<td>Slightly reduced; Reduced; TBD; TBD.</td>
<td>Monoclonal antibodies in Spacelab SLS-1 (Cogoli et al., 1993); Genetic expression by RT-PCR, secretion by monoclonal antibodies (II-2, II-2R), insertion in the membrane by immunofluorescence (II-2R).</td>
<td>The main cause of the inhibition of T cell activation is the strongly reduced expression of II-2R.</td>
</tr>
<tr>
<td><strong>Overall activation</strong></td>
<td>Mitotic index; „Windows of sensitivity“; „Threshold of sensitivity“.</td>
<td>Reduced by 90-70%; Probably yes, incidental interruptions of the 1xg centrifuge in space lead to lower activation than in 1xg ground controls, TBC. Probably yes, at 0.6xg activation is close to that at 1xg, but is depressed below 0.6xg, TBC.</td>
<td>Incorporation of (^3)H-thymidine into DNA, four Spacelab missions (SLS-1, D-1, SLS-1, IML-2), (Cogoli et al., 1984, 1993, Pippia et al., 1996); Interruption of the incubation at 1xg at given times by exposure to 0kg (Bechler et al., 1986, Pippia et al., 1996). incubation in a multi-g centrifuge in SLS-1 (Cogoli et al., unpublished data).</td>
<td>During the 72 h time required for full activation of T cells, there are critical phases lasting 10-60 min during which incubation at 0kg inhibits activation. There is a threshold of gravisensing between 0.6 and 0kg.</td>
</tr>
</tbody>
</table>

\(^a\)TBD: to be determined, TBC: to be confirmed in further investigations.

\(^b\)The expression of c-fos and c-myc is inhibited at 0kg in A431 cells upon activation with epidermal growth factor (de Groot et al., 1991).


