EFFECT OF GRAVITY CHANGES ON THE CYTOSKELETON IN HUMAN LYMPHOCYTES*

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ABSTRACT

A series of experiments performed in space as well as in models of low gravity conditions on ground have shown that 1) The mitogenic activation of human lymphocytes in vitro is severely depressed; 2) Binding of the mitogen Con A to membrane glycoproteins is unchanged, but patching and capping are slightly retarded; 3) Cell-cell interactions and aggregate formation are occurring, although space aggregates are smaller than the respective ground controls; 4) Single cells show autonomous movements with a higher velocity than at 1xg; 5) The amount of interleukin-2 receptor (IL-2R) and interleukin-2 (IL-2) measured as protein secreted in the medium is depressed; 6) The expression of both IL-2 and IL-2Rα genes is significantly inhibited; and 7) 1-2 % of genes monitored using cDNA microarray hybridization technology show significant modulation in response to short term low gravity conditions.

Changes in cell activation and signal transduction as well as cell movements and aggregate formation may be related to changes in the cytoskeleton. In fact marked alterations in the structure of the intermediate filaments of vimentin as well as in the microtubules network are observed in low gravity. Most evident is the appearance of large bundles in the vimentin structure; their number is significantly increased in the samples exposed to 30 seconds of low gravity compared to the in-flight 1 x g controls. These results indicate that direct effects of low gravity on the cytoskeleton in human lymphocytes are likely.

INTRODUCTION

Twenty years of research in true low gravity in space as well as in models of low gravity conditions on ground have clearly shown that human lymphocytes in culture are sensitive to gravity changes, as profound alterations of several cellular functions have been found (for reviews see: Cogoli, 1993; Cogoli and Cogoli-Greuter, 1997; Cogoli, 1997; Lewis 2002). It still must be questioned if the changes are really due to a direct effect of gravity unloading or if they are due to an indirect effect. Indirect effects are attributable to changes of the microenvironment of the cell due to lack of convection and sedimentation. Dramatic changes of the cytoskeletal structures in lymphocytes have been observed in low gravity (Cogoli-Greuter et al., 1998, Lewis et al., 1998). This suggests that single cells in culture may experience direct effects of gravity unloading. Whether this is true also for the other changes will be discussed below. This could be the case if (i) some of the cellular functions affected are related to the cytoskeletal network; and (ii) the changes in the cytoskeleton as well as the other effects are not due to the conditions of launch.

The cytoskeleton of mammalian cells is a network of microfilaments, composed of actin subunits, microtubules and intermediate filaments (Bershadsky and Vasiliev, 1988). In lymphocytes the intermediate filament is composed of vimentin. The three main cytoskeletal elements are interconnected by many hundreds of associated cytoskeletal proteins. These proteins also link the cytoskeletal elements to the cell membrane. The cytoskeleton plays an important role in the cell. It maintains cell shape, provides mechanical support, coordinates and directs cell locomotion as well as cytoplasmic streaming, maintains the organelles and cellular proteins in their proper spatial position with respect to each other and also plays a key role in signal transduction (Janmey, 1998). It is known that a disruption of filaments or of the filament association with related proteins has an impact on signal transduction, cell growth and metabolism (Valitutti et al., 1995).

Gravity unloading per se is not sufficient to change the shape of a cell because its microenvironment is subjected to forces that are many orders of magnitude larger than the force of gravity (Albrecht-Buehler, 1990; 1991). However, organelles with a higher density than the cytoplasm (like ribosomes, centrioles, nucleolus) may exert at 1xg some pressure on cytoskeletal and other structures. This pressure would disappear in low gravity. The cytoskeletal filaments are subjected to dynamic and rapid polymerization and depolymerisation processes. Tabony et al. (2002) have discovered that the in vitro selforganisation of microtubules in cell free solutions does not occur in low gravity. This finding leads to the conclusion that it might well be that irregularities in polymerization of cytoskeletal structures can have severe implications at least on some of the different cellular aspects so far studied in space.

Human peripheral blood lymphocytes – the cells of our immune system – are peculiar cells. They circulate in the blood but are also migrating through the endothelial membrane into the tissue. In circulation they are subject to repeated hydrodynamic and mechanical stress and therefore need a cytoskeleton that is sufficiently rigid to protect them from damage. In circulation lymphocytes are round shaped. Migration from circulation into tissue requires the rapid conversion from a semirigid to a highly deformable state, as cells must undergo extensive shape changes to penetrate the narrow interstitial spaces between endothelial cells (Anderson et al., 1997). During transendothelial migration, lymphocytes become highly polarized by developing a leading edge and a distinctive tail like trailing edge or uropode. This process is driven by rapid reorganization of all three cytoskeletal filament

**I dedicate this publication to the crew of STS-107*

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systems. The leading edge is distinguished by one or more filamentous actin rich pseudopodia. As the uropode forms, both the microtubules and vimentin intermediate filaments retract and become concentrated within it (Brown et al., 2001). Similar shape changes are occurring when cells locomote or crawl over a surface. Thus the cytoskeleton has a special role in lymphocytes.

Lymphocytes can be easily isolated as resting cells from peripheral blood of healthy donors. By the addition of an activator – either an antigen or mitogen - the T-cells can be transformed in vitro from resting into activated (dividing) cells. The mitotic index is determined by biochemical methods. During activation, T cells produce a number of lymphokines.

Since 1983 we have studied several aspects of the in vitro activation of human lymphocytes in true low gravity in the Spacelab and on sounding rockets as well as in models of low gravity conditions in the fast rotating clinostat and in the random positioning machine. In this paper I will review the most important findings made by our research team. Furthermore I will describe the role of the cytoskeleton in lymphocyte activation. It is not the scope of this article to give a review of all data found by other research teams.

ACTIVATION AND SIGNAL TRANSDUCTION

In the early 70-ies several investigators reported that lymphocytes from the majority of crewmembers on US and Soviet missions had a decreased response to mitogens after space flight (Konstantinova et al., 1973). While it was known at that time that stress might depress the immune system, it was not clear whether microgravity as such might directly influence the cells involved.

Stimulated by these findings we have studied aboard Spacelab 1 for the first time the in-vitro activation of human lymphocytes in low gravity. Cultures of purified lymphocytes were activated in flight by the addition of the T cell mitogen concanavalin A (Con A) and incubated for 3 days at 37°C. After the addition of radioactive thymidine and incubation for another 2 hours, the samples were fixed in space and analysed later on ground. The degree of activation was measured as the amount of radioactive thymidine incorporated into the DNA of the cells. The result was quite surprising (Cogoli et al., 1984). Lymphocytes exposed in low gravity conditions to the mitogen Con A showed less than 3% of activation compared to the synchronous ground control performed in the ground laboratory at Kennedy Space center.

More sophisticated experiments followed in Spacelab D1 in 1985 (Bechler et al., 1986; Cogoli et al., 1988), in SLS-1 in 1991 (Cogoli et al., 1993) and in IML-2 in 1994 (Pippia et al., 1996). In all 3 experiments controls were incubated in an inflight 1 x g reference centrifuge. All investigations confirmed the effect (Figure 1). The historic experiment on Spacelab 1 has triggered an intensive research by us and other teams to explain the cause of the impaired activation.

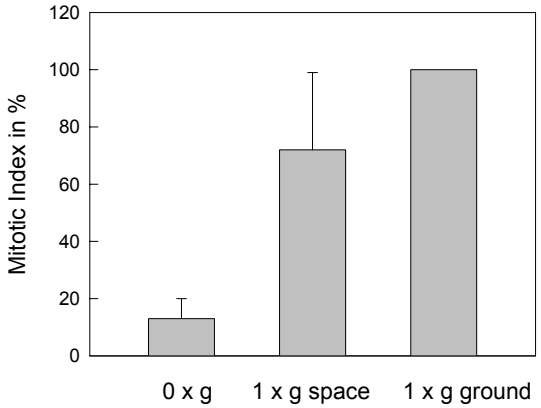

Figure 1: In-vitro activation of human lymphocytes by Con A. Summary of results obtained on three different Spacelab missions.

The mechanism of T-cell activation is very complex and is based on three distinct pathways starting from the T cell receptor (TCR)-CD3 complex, from the CD28-B7 interaction (accessory cells) and from the autocrine interleukin-2IL(IL-2)/IL-2 receptor (IL-2R) interaction respectively (Figure 2) (Schwarzenberg et al., 2000). Three signals are required for full T cell activation (Crabtree and Clipstone, 1994). The first signal is delivered to the TCR/CD3 complex either by the antigen presenting cell (antigen fragment + major histocompatibility complex, MHC) or by anti-CD3, or by the mitogen Con A. Con A does not bind to a specific receptor but rather to glycoproteins fitted with α-glycosides (see below). The second signal is a co-stimulatory signal delivered either by the accessory cells (usually monocytes) via B7/CD28 interaction or by anti-CD28, or, according to other authors, by interleukin-1 (IL-1). Upon interaction with T lymphocytes, the accessory cells secrete interleukin-1 (IL-1). The third signal is delivered by IL-2 secreted by the T cell itself and recognized by the IL-2R. Both IL-2 and IL-2R are expressed during the activation process following transduction of the first two signals (Foletta et al., 1997; Leonard and O'Shea, 1998). The signaling cascade involves protein kinases, GTPases, second messengers and transcription factors; a key role plays the Phosphokinase C consisting of many isozymes.

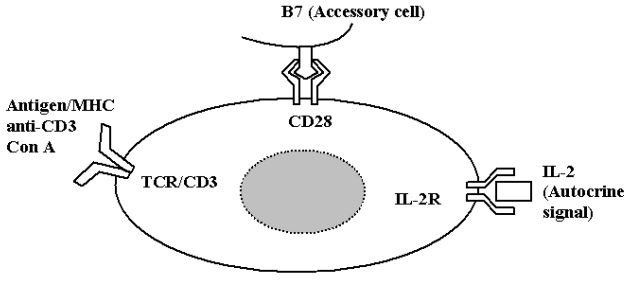

Figure 2: The three signals required for complete T cell activation (From Schwarzenberg et al., 2000).

The first signal: Binding of mitogen

One of several possible explanations of the depression of the in vitro activation of human lymphocytes by Con A is that the spatial orientation of the Con A receptors on the membrane may be altered under low g conditions, thus altering the binding of the mitogen. This hypothesis has to be seriously considered since changing g-level may not only alter cell shape but also the configuration of the membrane.

The mitogen Con A, a lectin carrying four binding sites specific for α-glucosides, is a specific activator of T-lymphocytes. Its binding to the glycoproteins of the cell membrane brings about the formation, first, of patches and, secondly of caps of Con A receptors. Patching, i.e. the formation of clusters of Con A receptors on the cell membrane is an energy-independent step. Capping, i.e. the formation of one cluster of Con A receptors at one pole of the cell occurs only at 37° C and is an energy-dependent step. Capping is also a necessary step toward lymphocyte activation. Patching and capping are occurring within a few minutes at 37°C under 1 g conditions. Abnormalities in this process would obviously alter the transduction of the first activation signal.

To study the binding of Con A as well as patching and capping, we have performed a series of investigations on sounding rockets. Fluorescent-labeled Con A was added to the cells in culture as soon as low gravity conditions were established. The cells were fixed in low gravity either at different preset times after the addition of the mitogen or before the end of the low g phase. A control experiment was performed on ground or in an in-flight 1 x g centrifuge.

In a first experiment on MASER 3 in 1989 no significant differences in the rate of binding; patching and capping compared to the 1 x g ground control were observed (Cogoli et al., 1990). Binding was very fast and completed after 30 s.

The second experiment was carried out on MASER 4 (1990) to test if the lack of differences between flight and 1 x g control samples was due to a lack of influence of low gravity or to the fact that the cells did not have sufficient exposure time to "adapt" to low gravity. Therefore, Con A was injected into the samples 3 and 5 minutes after the onset of low gravity. The data revealed that even a 5-minute exposure of the cells to low gravity prior to the addition of Con A does not affect the binding of the mitogen to the cell membrane compared to the ground control (Cogoli et al, 1992). Furthermore there are no significant differences in patching after the cells have been exposed for 3 minutes to low gravity before addition of the mitogen. However, patching is significantly retarded when Con A is added after 5 minutes of low gravity exposure. In the flight samples, fixed after 90 seconds incubation with Con A, 2.9±0.9% of the cells show patching compared to 7.4±3.4% in the ground control. Thus patching is clearly retarded after the cells have been exposed for 5 minutes to low gravity before the addition of Con A.

The percentage of cells showing patching and capping is increasing with the incubation time after the addition of Con A. Therefore the experiment on binding, patching and capping was repeated on MAXUS 2 taking advantage of the longer low gravity time (12 minutes) compared to the MASER flights (5-6-minutes). An in-flight 1 x g centrifuge allowed to discriminate between effects of low gravity and effects of launch. Instead of lymphocytes we used Jurkat cells - a human leukemic T cell line (Cogoli-Greuter et al., 1998). The analysis shows that binding of Con A to Jurkat cells is not influenced by gravity changes (Figure 3). Patching of the Con A receptors, however, is significantly lower in low gravity compared to the in-flight 1 x g control as well as the ground control. The results confirmed thus the previous findings obtained on MASER 3 and 4. They also showed that patching is a dynamic process also under low gravity conditions that will be completed with longer incubation. Despite the longer low gravity time, the number of cells showing capping was too low for a statistical evaluation. From these observations we concluded that the influence of low gravity on the delivery of the first signal of activation is rather little and that, therefore, rapid processes like binding of the mitogen, patching (although slightly retarded) and probably also capping are not involved in the depression of the in vitro activation of T lymphocytes observed in several Spacelab experiments.

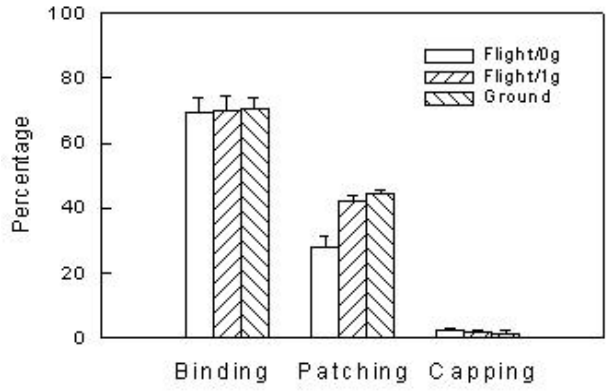

Figure 3: Binding of Con A to Jurkat cells followed by patching and capping of the Con A receptors. The bars represent the percentage of cells showing either binding, patching or capping. Standard errors of the means are given.

The second and third signal

The second signal necessary for the in vitro activation of lymphocytes by the mitogen Con A is provided by IL-1 delivered by the monocytes acting as accessory cells and always present in small amount in preparations of peripheral blood lymphocytes. The third signal is IL-2 synthesized and secreted by the T-cells and binding to its receptors (IL-2R) on the cell membrane.

In investigations performed during SLS-1 and IML-2 we have shown that the amount of IL-2R measured as protein secreted in the medium is strongly depressed. Furthermore also the secretion of IL-2 is reduced, whereas IL-1 is enhanced (Cogoli et al., 1993; Pippia et al., 1996). These findings support the hypothesis that an

alteration of the IL-2/IL-2R function may be the cause of the observed depression in low gravity. This can be due either to a reduction of genetic expression, or of the secretion of IL-2 and its receptor IL-2R, or to a failure to insert the receptor into the membrane. Indeed it has been observed that IL-2R is not expressed on the surface of cells activated in low gravity (Hashemi et al., 1999). The delivery of the second signal - IL-1 - is not affected.

To test this hypothesis we have studied the genetic expression of IL-2 and its receptor in Con A activated lymphocytes with the RT-PCR technology. Low gravity conditions were modeled in the random positioning machine (Walther et al., 1998). The data show that the onset of the genetic expression of IL-2 in Con A activated cells begins approximately 1 hour after exposure to the mitogen at 1 x g as well as in models of low gravity conditions. However there is a clear inhibition at low gravity (Figure 4). The IL-2 receptor consists of 3 protein chains. We have studied the expression of the α- and β-chains only, because the γ-chain is constitutively expressed. The expression of the α-chain is significantly inhibited in modeled low g, whereas the β-chain is not affected (Figure 5). These results clearly demonstrate that the non-responsiveness of lymphocytes in the in vitro activation in low gravity is due to a dysfunction of the transcription of the IL-2 and IL-2Rα-gene. On the other hand, the expression of the IL-1 gene was enhanced. This excludes also the possibility that the reduction in the expression of the other genes was due to a mechanical damage of the cells in the random positioning machine or in the fast rotating clinostat. The fact that we observed a selective inhibition of the IL-2Rα gene but not of the IL-2Rβ gene supports the notion of a direct effect of gravity unloading. An experiment performed on flight STS-107 to confirm in true microgravity the data obtained on ground was lost in the catastrophe of Columbia in February 2003.

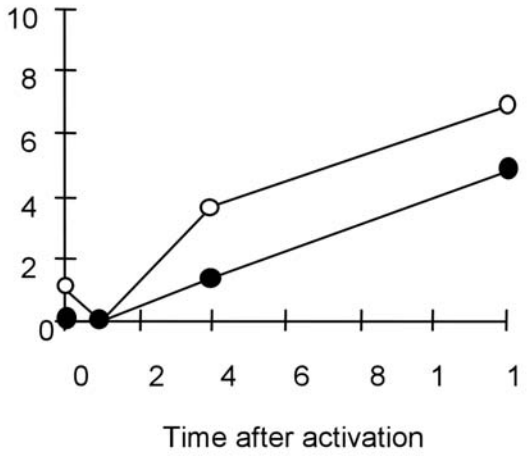

Figure 4: Effect of models of low gravity conditions on the genetic expression of IL-2. *The data shown are from one experiment in the fast rotating clinostat and are consistent with another on the fast rotating clinostat and with three analogous experiments on the random positioning machine. Open dots: 1 x g, black dots: modeled low g. (From Walther et al., 1998).*

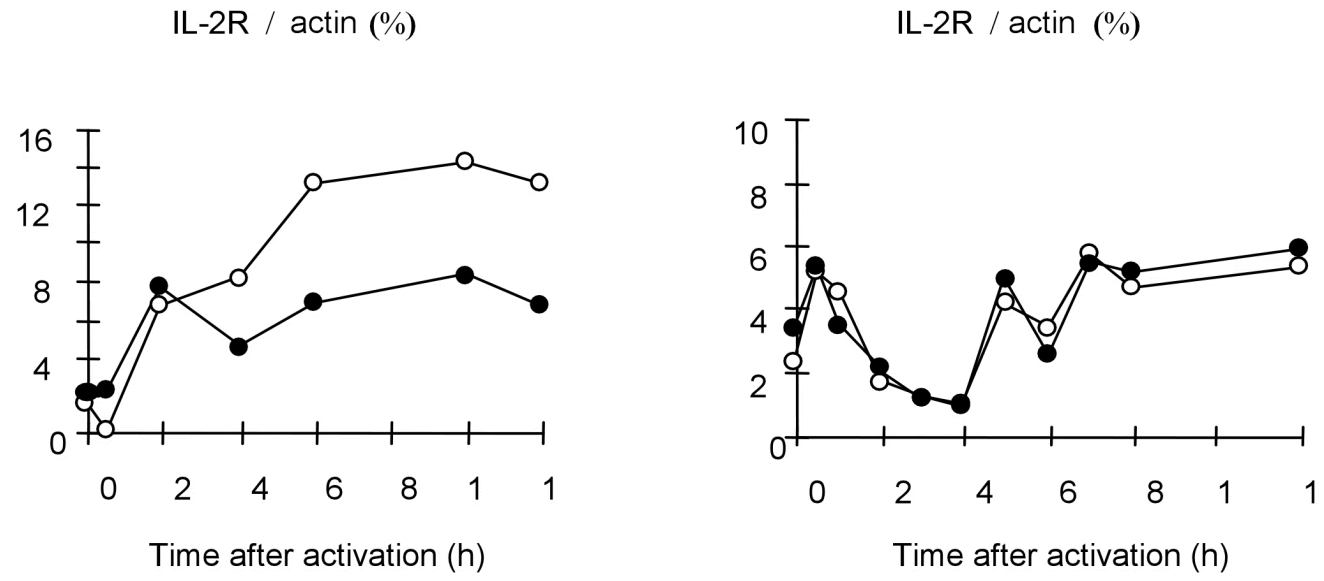

Figure 5: Effect of models of low gravity conditions on the genetic expression of IL-2Rα (left chart) and IL-2Rβ (right chart). *The data shown are from one experiment on the random positioning machine and are consistent with 4 analogous experiments on the random positioning machine for IL-2Rα and another analogous experiment in the fast rotating clinostat for IL-2Rβ. Open dots: 1 x g, black dots: modeled low g. (From Walther et al., 1998).*

On a sounding rocket flight (MASER 9) we have studied the early gene expression within the first 5.5 minutes after the addition of the mitogen. PBL were activated by Con A at the onset of the low gravity phase and lysed before its end. The control experiment was performed in an in-flight 1 x g centrifuge in order to discriminate from launch effects known to trigger gene expression. The transcriptional response was monitored by the cDNA microarray hybridization technology. We found that in T lymphocytes from two different donors about 1-2% of the genes monitored show significant modulation in response to low gravity (Negri et al., 2003). The expression of the IL-2Rα gene is clearly depressed whereas the IL-1 gene is induced already within 5.5 minutes in low gravity compared to the in-flight 1 x g control.

Alternative signaling pathways

Preliminary work in our laboratory has shown that activation is not depressed when purified T cells are activated in the presence of anti-CD3 and IL-2 in the random positioning machine. This is an indication that different signaling pathways may be affected differently (Schwarzenberg et al., 2000). Cooper and Pellis, 1998, reported that the loss of activation of T cells in models of low gravity conditions, in the rotating wall vessel, was restored by direct activation of PKC with phorbol myristate.

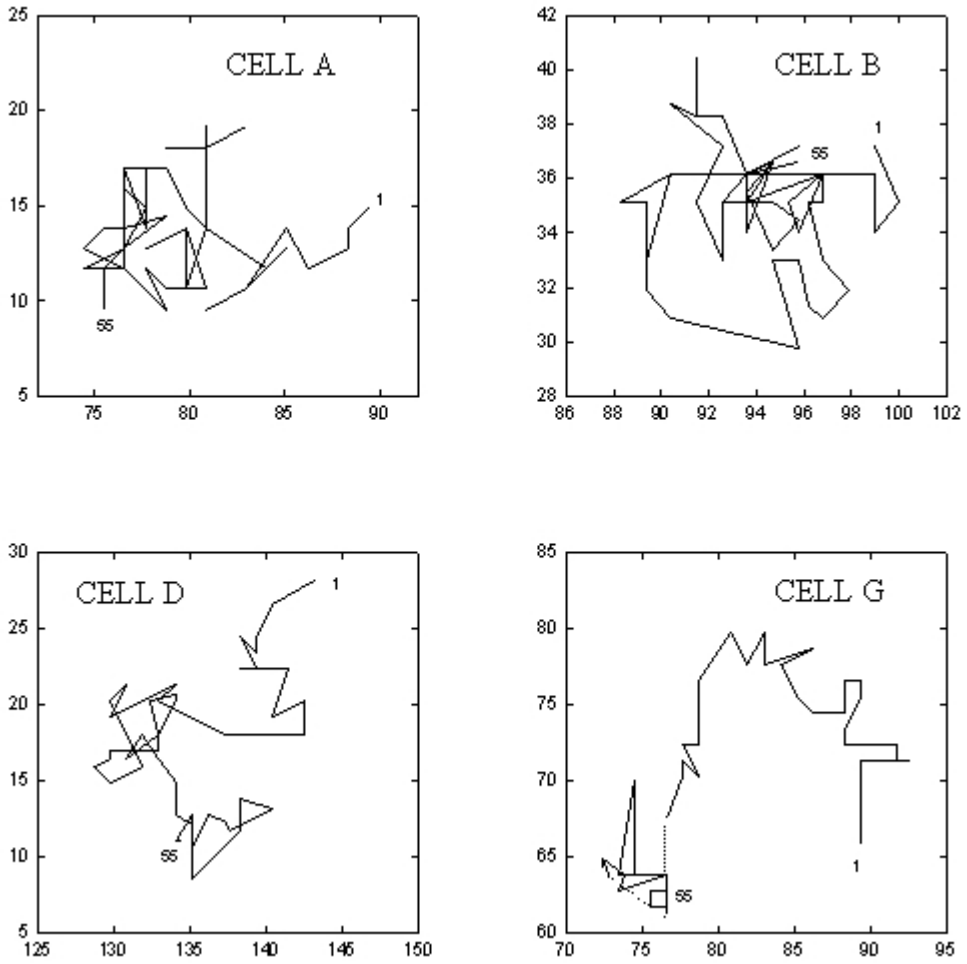

Figure 6: Motility of human lymphocytes in microgravity: Detailed motion behaviour of 4 cells (1 measuring point each 13 seconds). The numbers on the axis, in μm, correspond to the position of the cell in the viewing field

CELL MOTILITY AND CELL-CELL INTERACTION

Cell-cell interactions and aggregate formation are important means of cell communication and signal delivery in the mitogenic T lymphocyte activation. From earlier experiments we had indirect evidence that cells are moving and interacting also in space as aggregates were observed in samples fixed in space (Cogoli et al., 1984; 1988).

On the sounding rocket MAXUS 1b we studied the movements of free floating non-activated peripheral blood lymphocytes in real time in low gravity conditions. For this purpose we had a microscope combined with a video camera in the experiment module, which could be

operated by telecommand from ground (Cogoli, 1993). The images recorded under low gravity show that the free-floating non-activated cells are able to display autonomous motion in random directions (Cogoli-Greuter et al., 1998). A detailed analysis shows that the movements are much more complex (Figure 6). The cells often change direction, move back and forwards and sometimes cross the same point several times. The average velocity, calculated from the displacement in 13 seconds increment, is 8.4 ± 1.2 μm/minute, with a range of 0 - 29.4 μm/minute. Of interest is also the observation that the cells in low gravity were not all round-shaped. Very often they exhibited longitudinal forms, rotated around their axis and also showed contraction waves similar to those described in the literature for lymphocytes that move in collagen gels under 1xg conditions (Haston and Shields, 1984). All 11 cells in the observation field showed motion capability under low gravity conditions. This is in contradiction to the behaviour of lymphocytes under 1xg conditions, where mostly activated cells only or cells in the presence of a chemo attractant show this capability (Wilkinson, 1987).

In the IML-2 mission we studied the motility and aggregate formation of human peripheral blood lymphocytes during the in vitro activation. After addition of the mitogen Con A, a few hours after launch, the cells were incubated at 37°C in the Biorack incubator and observed 3 times for 30 minutes during the 3 days of activation in the NIZEMI-facility (Friedrich et al., 1996). NIZEMI had a microscope and a video camera. Real time video was down linked to the investigator on ground for short time and than recorded in space. The control experiment was performed on ground. An evaluation of the videotapes reveals that lymphocytes resuspended in low gravity in the presence of the mitogen Con A form aggregates (Figure 7) (Cogoli-Greuter et al., 1996). Aggregates can be observed at 1 x g as well as in low gravity already 12 hours after the addition of the mitogen. The space aggregates observed 78 hours after the addition of Con A were larger, i.e. consisted of more cells, than the one after 12 and 48 hours. Thus we have demonstrated that cell-cell interactions are occurring at 0 g. In the second and third observation period the ground aggregates were mostly larger than the corresponding space aggregates. The aggregates, both in space as well as on ground, change their shape throughout the observation period. Single cells in suspension not attached to an aggregate exhibit motions similar to those previously observed in non-activated cells on a sounding rocket flight. They also show rapid changes from round-shaped to longitudinal-shaped cells and vice-versa, rotate around their axis and show contraction waves. Their morphology observed by electron microscopy is typical for moving cells. The mean velocity of the free cells is significantly higher in low gravity, than at 1 x g and does not change with increasing incubation time. Conversely, at 1 x g the mean velocity of cells decreases significantly (Table 1). This behaviour is normal and correlates with the finding that Con A activated cells are significantly less motile in the late S and G_2 + M phases than non-activated cells (Ratner et al., 1988). The fact that at 0 x g the mean velocity of free cells does not decrease with incubation time is an indication that these cells do not proceed through the S and G_2 + M phases of the cell cycle and thus do not proliferate as observed in several investigations in space.

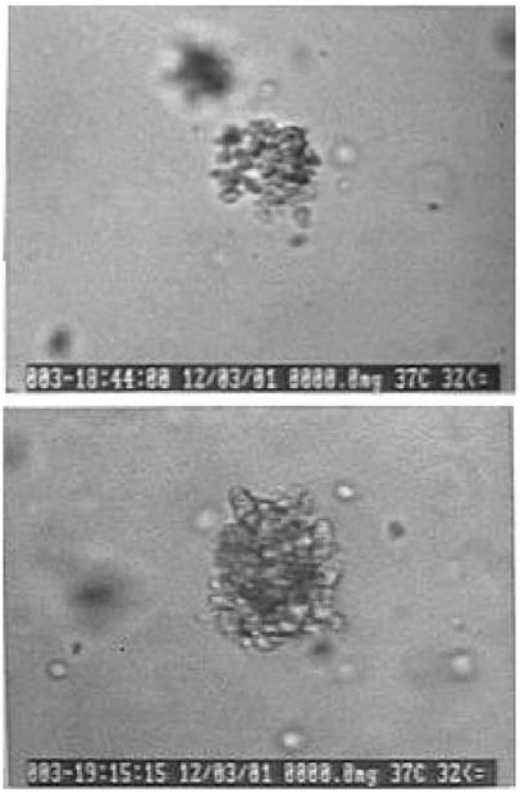

Figure 7: Two different "space" aggregates 78 hours after the addition of Con A.

Hours after addition of Con A	Space	Ground
12	6.6 ± 1.4 (0 – 23.0)*	2.3 – 1.1 (0 – 13.4)*
78	6.7 ± 1.6 (0 – 23.0)*	0.9 – 0.8 (0 – 5.1)

Table 1: Average velocity (in μm min $^{-1}$) of single cells activated with Con A calculated from the displacements in the intervals of 30 seconds (mean ± S.E.M.).
**) extreme values*

CYTOSKELETON

So far only a few experiments have been done on the influence of gravity changes on the cytoskeleton.

In two experiments on the sounding rockets MAXUS 1 and 2 we studied the influence of gravity changes on the cytoskeletal structure of vimentin in Jurkat cells. On MAXUS 1 the cells were fixed at different times after the onset of low g. A control experiment was performed on ground. On MAXUS 2 Con A was added to the cells at the beginning of the low gravity phase. The cells were than fixed before the end of this phase. On this flight we had an in-flight 1 x g centrifuge for the control experiment (Cogoli-Greuter et al., 1998). The morphological analysis by fluorescence microscopy revealed dramatic changes of the structure of vimentin already after 30 seconds of exposure to low gravity (Figure 8). Most evident is the formation of thick bundles in low gravity compared to the fine network in the 1 x g control. The other significant alterations consist of formation of aggregates of proteins, appearing as fluorescent points and suggesting depolymerisation processes, as well as of discontinuities of the filamentous network. We were well aware of the fact that the changes may be due to launch stress. However, the results of MAXUS 2 where the control was in the in-flight 1 x g centrifuge confirmed the findings (Figure 9). The binding of Con A to the cell membrane at the onset of the low g phase had no influence on the changes in the cytoskeletal structure of vimentin.

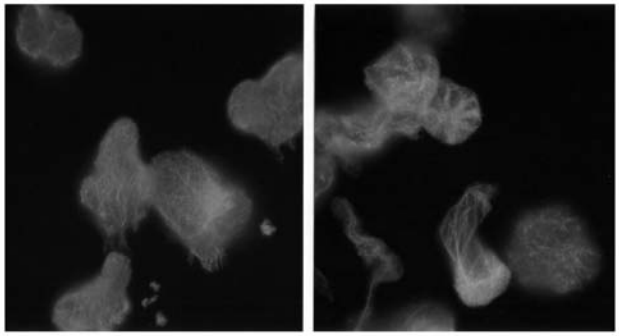

Figure 8: Cytofluorographs of the intermediate filaments of vimentin in Jurkat cells; *Left: ground control, Right: flight sample fixed after 30 seconds in low gravity (Photo by L. Sciola).*

Vimentin plays an important role in vital mechanical and biological functions such as cell contractility, migration, stiffness, stiffening and proliferation (Wang and Stamenovic, 2002). For many years the role of vimentin in lymphocytes was unclear. First reports showed that vimentin in B cells co-capped with membrane immunglobulins upon anti Ig stimulation (Dellagi and Brouet, 1982). In T lymphocytes, activation with Con A resulted in altered distribution of vimentin which was correlated with specific phases of the cell cycle indicating that the function of vimentin may change as cells traverses through the cell cycle (Paulin-Levasseur and Brown, 1987). Recently it was shown that the vimentin intermediate filaments are the dominant cytoskeletal element in determining the rigidity of circulating lymphocytes (Brown et al., 2001). The collapse of the vimentin filaments during polarization is necessary to achieve the increased cell deformability required for transendothelial migration. Microtubules in contrast are not sufficient to maintain rigidity. Furthermore it was also discovered that Plectin, a huge protein associated with intermediate filaments is expressed in lymphocytes. Vimentin, Plectin and Fodrin are physically interconnected in lymphocytes; this "VPF" assembly spans all the way from the nucleus to the plasma membrane and condenses into the uropod within 1 minute of chemokine stimulation (Brown et al., 2001).

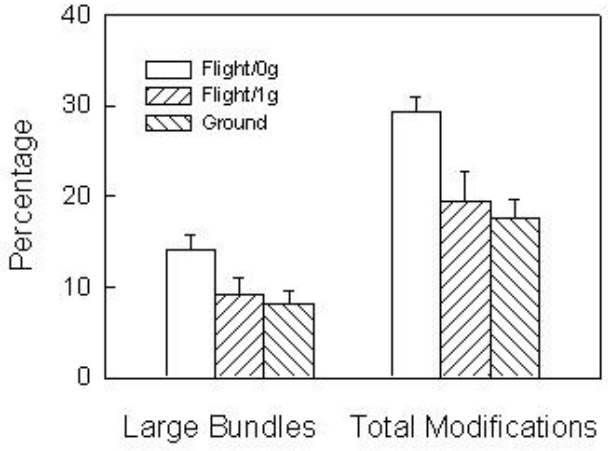

Figure 9: Influence of gravity changes on the structure of vimentin in Jurkat cells. *The bars represent the percentage of cells showing either the appearance of large bundles or the total of modifications. Standard errors of the means are given.*

Lewis et al. (1998) found that also in the tubulin structure dramatic changes are occurring in Jurkat cells exposed to low gravity: The microtubule organizing center was disorganized, the microtubules coalesced and were not extending to the cell periphery. However, the microtubule reorganized within 24 hours; but the cells did not proliferate. Furthermore they found that the vibrations of the shuttle launch simulated on ground disrupts the microtubule organizing center and the microtubules in a similar way as observed in the space experiment (Cubano and Lewis, 2001). However, in contrast to the space cells, the vibrated cells resumed active growth. The authors concluded that growth arrest is not a direct result of cytoskeletal disruption caused by vibration. But it is unclear whether the microtubules reorganize properly despite the fact it seems so. A significant increase in the genetic expression of plectin and other cytoskeletal elements was observed by Lewis et al (2001) in space flown Jurkat cells after 48 hours compared to ground controls. As mentioned above, Plectin is a versatile cytoskeletal linker providing mechanical strength and structure by interconnecting intermediate filaments, microtubules and actin to each other and to the cell membrane. Therefore, these findings may imply that cytoskeletal elements, made de novo, are not properly reassociated.

ROLE OF CYTOSKELETON IN LYMPHOCYTE ACTIVATION AND MOTILITY

Involvement in mitogen binding and downstream signaling

First reports in the early 70ies have shown that the actin cytoskeleton might be involved in the cap formation (de Petris and Raff, 1973). In the last few years many papers have been published on the involvement of the cytoskeleton in the aggregation of the receptors on the cell membrane and the transduction of the signals to the nucleus (for reviews see: Penninger and Crabtree, 1999; Bauch et al., 2000; Acuto and Cantrell, 2000). Although it is still a matter of controversy some facts seem to be clear today.

The cap, i.e. cluster of Con A receptors on one pole of the cell, includes many molecules that are involved in lymphocyte activation. Therefore it was thought that the cap might be required for conveying signals to the cell interior. But the significance of the cap and its role in signaling was called into question, as cap formation requires several minutes, whereas tyrosine phosphorylation, typical of early activation events, occurs within 10 s of antigen receptor engagement. But today it is generally accepted that the cap is necessary for signal transduction. The forces driving the formation of caps require actin polymerization in the T-cell (Wulfing et al., 1998). After engagement of the receptor some first signaling molecules are activated and start actin polymerization and cap formation. In a next step important signaling molecules are transferred into the cap. This leads to an amplification of the signals and finally to cell proliferation. A key enzyme that is translocated to the cap is the PKCθ, whereas the other PKC isozymes remain in other regions (Monks et al., 1998). Thus, PKCθ is a candidate for an effector kinase that links the cap to the downstream signaling pathways. PKCθ also provides a survival signal, which protects T cells from apoptosis (Altman and Villalba, 2003). It is interesting to note that in low gravity (3 different shuttle flights) (Lewis, 2002) as well as in models of low gravity conditions (Maccarrone et al., 2003) an increase in the number of apoptotic Jurkat cells was found. In order to get a physiological response, i.e. proliferation, distinct signaling pathways must be activated in a temporally and spatially coordinated action. Without the formation of caps individual signaling pathways may function, but the synergy is lost. Receptor clustering in a cap is therefore important for assembling signaling molecules at focal sites. Only this than promotes sustained signaling necessary for lymphocyte activation. We have observed that patching is slightly retarded in low gravity. Furthermore we also have found that dramatic changes in the structure of vimentin occur after very short exposure time to low gravity. Thus it cannot be excluded that the polymerization of actin is disturbed. But so far we have no experimental evidence for this. The fact that increased apoptosis was observed in low gravity might be an indication that the location of the PKCθ is altered.

The cytoskeleton not only plays a key role in clustering the signaling molecules near the membrane. Actin and its associated proteins as well as microtubules and vimentin are described to be involved in the downstream signaling. For an effective signaling all enzymes must be confined near their respective substrate. This is achieved by a scaffold formed by the different cytoskeletal elements (Penninger and Crabtree, 1999). Evidence that an intact cytoskeleton is required for proper signal transduction is provided, in part, from studies showing that signal transduction can be blocked by drugs that alter the cytoskeleton. Thus the cytoskeletal structure and scaffold geometries can directly regulate the molecular dynamics of signaling and biochemical responses. Furthermore there is increasing evidence of a functional association between the cytoskeletal protein spectrin and PKCβ in the cytoplasm of lymphocytes (Gregorio et al., 1994). PKCβ plays a critical role in lymphocyte activation related signaling. A disorganization of the cytoskeleton due to gravity unloading could result in a disturbed localization of signaling molecules. Indeed, Schmitt et al. (1996) have found that the distribution of PKC is altered in low gravity. We have found that the expression of the IL-2Rα gene is inhibited, whereas the expression of IL-2β is not disturbed. This demonstrates that some of the signaling pathways are selectively affected.

Involvement in cell motility

In order to start locomotion on a surface or also transmigration through the epithelium a resting lymphocyte changes its round shape into a polarized one. This involves a reorganization of the cytoskeletal network with a collapse of the vimentin system, which retracts, into the uropode (Brown et al., 2001). During locomotion, the cytoskeletal structures are subjected to repeated cycles of reassembly processes. We have found that in the absence of gravity suspended cells, either non activated, or activated by the mitogen Con A, show a moving behaviour and a polarized morphology similar to those observed at 1 x g. Instead of crawling on a surface under 1 x g conditions, in low gravity the cells swim in the culture medium. Furthermore we have observed rapid changes in the structure of vimentin upon gravity unloading, in particular the formation of thick bundles but also protein aggregates suggesting depolymerisation processes. Dramatic changes have also been detected in the microtubule network (Lewis et al., 1998). These changes may induce the motility of lymphocytes under low g conditions by contraction and elongation of the free-floating cells.

CONCLUSION

Twenty years of space research have clearly shown that important cellular functions as signal transduction and cell proliferation in human lymphocytes are severely affected upon mitogenic activation by Con A. Lymphocytes – inactivated or activated – were found to be highly motile in low gravity. The cytoskeleton plays an important role in signal transduction and cell motility. The fact that remarkable changes in the cytoskeletal structures of vimentin and tubulin have been observed is an indication that the cytoskeleton may be responsible in part also for the other changes. Many more experiments

must be performed in real, but also in models of low gravity conditions to confirm this hypothesis.

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