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ORIGINAL ARTICLE

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Hypergravity speeds up the development of T-lymphocyte motility

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Abstract The effect of altered gravity on single cells has been reported in a number of studies. From the investigation of the immune system response to spaceflight conditions, interest has focused on the influence of gravity on single lymphocytes. Microgravity has been shown to decrease lymphocyte activation and to influence motility. On the other hand, the effect of hypergravity on lymphocyte motility has not been explored. We studied the migration of human peripheral blood T lymphocytes cultured in vitro in a hypergravity environment (10*g*). After hypergravity culture for 1–11 days, T cells were seeded on a fibronectin-coated glass surface, observed by time-lapse bright-field microscopy, and tracked by a computer program. We found that T cells, activated and then cultured in hypergravity, become motile earlier than cells cultured at normal gravity. These results suggest that hypergravity stimulates T cell migration.

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Introduction

The question of how the functions of the human body are altered by spaceflight conditions has focused interest also on the study of how the gravity force affects single cells. Such an investigation sheds light on fundamental aspects of the physics underlying cell behaviour. Besides cell–substrate and cell–cell contacts, a variety of other physical aspects are involved in cell culture when considering the changes in gravity, such as convection, flocculation, coalescence, interfacial tension, and hydrostatic pressure (Todd 1989).

Human lymphocytes have been studied extensively in vitro in conditions of microgravity, either in spaceflight or in modelled microgravity. Lymphocytes have no specific organelle for gravisensing; moreover, the gravity force is negligible at the cellular length scale when compared to the typical intramolecular forces. Yet, spaceflight cultivation has been shown to induce dramatic effects on lymphocyte activation (Cogoli et al. 1984; Pippia et al. 1996), signal transduction (Cogoli 1997; Schwarzenberg et al. 2000), cytokine secretion (Cogoli and Cogoli-Greuter 1997; Walther et al. 1999), apoptosis (Lewis et al. 1998), and cell–cell interactions (Cogoli-Greuter et al. 1996). Changes in the expression of molecules and in cytoskeletal structure have also been reported (Cogoli-Greuter et al. 1994; Cogoli 1997; Walther et al. 1998; Lewis 2004; Cogoli-Greuter 2004).

Similar effects can be reproduced on the ground in modelled microgravity by means of bioreactors (Pellis et al. 1997; Cooper and Pellis 1998; Hashemi et al. 1999; Cooper et al. 2001; Risin and Pellis 2001; Sundaresan et al. 2002). Exposure to modelled microgravity reduces lymphocyte migration through gels, to an extent dependent on the duration of exposure to microgravity and on the preactivation of the cells (Pellis et al. 1997; Sundaresan et al. 2002). In particular, non-activated lymphocytes lose their migration capability and are not

able to recover it even after a period of culture in normal gravity. On the other hand, direct observation of cells in suspension in true microgravity shows that lymphocytes are motile and have even higher velocities than on the ground (Cogoli-Greuter et al. 1996).

Similar to microgravity, hypergravity has been found to influence T cell activation and proliferation (Tschopp and Cogoli 1983; Lorenzi et al. 1986, 1988). Yet, the effect of hypergravity on T cell motility has not been studied before. This work focuses on the effects of hypergravity (10*g*) culture on T cell migration. We used time-lapse microscopy to classify and quantify the locomotory behaviour of T cells. We found that hypergravity acts as a stimulus for motility, making activated T cells become motile earlier than cells cultured in normal gravity.

Materials and methods

Experimental procedures

Cell preparation

Separation, purification, and culture of T cells Peripheral blood mononuclear cells (PBMC), were obtained from human peripheral blood of healthy donors by stratification on Ficoll-Hypaque (Nycomed, PHARMA AS, Oslo, Norway). T cells were then obtained by rosetting with sheep red cells. T cells were cultured in RPMI 1640 culture medium (VLE RPMI 1640, Biochrom AG, Berlin, Germany) +10% foetal bovine serum (HyClone, Logan, UT) and kept in an incubator at 37°C, 5% carbon dioxide. The presence of other mononuclear cells was estimated to be below 5%, decreasing in time after activation.

Activation Polyclonal activation was obtained with phytohemagglutinin (PHA; Gibco Life Technology, Gaithersburg, MD) at 0.1% v/v, and interleukin-2 (IL-2; Eurocetus, Milano, Italy) at 20 units/ml.

Hypergravity culture T cells were incubated in 25 cm^2 culture flasks (Corning Incorporated Life Sciences, Acton, MA) spinning in a homemade centrifuge at 10*g* (300 rpm). The centrifuge, kept at 37°C and 5% CO_2 inside an air-flux incubator, rotated around a vertical axis. The flasks where positioned vertically in the centrifuge so that when spinning the cell solution distributed along what is usually the "bottom" surface of the flasks. Due to the geometry of this arrangement, there was a difference in the gravity field between the centre and the edges of the flasks. This difference was about 3%; however, it was sufficient to cause the accumulation of cells in the flask edges. For every measurement, a control sample was kept at 1*g* for the same amount of time in the same conditions and in the same kind of flasks, tilted at a small angle in order to make the 1*g* cells accumulate in the edge as well.

Washing and slide preparation T cells were washed before seeding by centrifugation for 10 min at 1,400 rpm (=200*g*) and then resuspended in fresh medium. T cells were seeded on fibronectin-coated coverslips (BD, Franklin Lakes, NJ) and observed immediately.

Cell count, proliferation, and viability Cell count (number of cells/ml) was performed manually with a haemocytometer. Viability was determined by propidium iodide uptake by using a BD-LSR flow cytometer (BD Biosciences, San Jose CA). Proliferation for activated cells was determined as previously described (Cosmi et al. 2003). Briefly, T cells (1×10^5 cells/well) were seeded in a 96-well plate; after 8 h of pulsing with 0.5 Ci (0.0187 MBq) 3H-TdR/well (Amersham), cultures were harvested and radionuclide uptake measured by scintillation counting.

Time-lapse microscopy

Motility experiments were performed by acquiring time-lapse images of T cells migrating on a fibronectin-coated surface. We used a homemade inverted transmission microscope, equipped with a water immersion objective (final magnification 20×, field of view ~300×200 μm^2). Images were acquired with a CCD camera and stored in a PC. The microscope stage, kept at 37°C, was moved by PC-controlled motorized translators. In experiment A (see Results), time-lapse sequences were taken for 3 h, at a rate of 1 frame every 30 s. In order to observe a large number of cells, for every frame we acquired a 4×4 matrix of images by translating the stage in steps of 300 μm in the *x* direction and of 200 μm in the *y* direction. Complete frames were composed afterwards. In successive experiments (B, C, and D) time-lapse sequences were acquired for 1 h and with a 5×5 matrix of images. We verified that the motility index (see below) does not change significantly if the observation time is reduced from 3–1 h (the relative variation is a few percent).

Motility data analysis

Cell classification

We divided the cells into the following groups with respect to their locomotory behaviour:

- Moving cells, i.e. cells migrating on the slide surface, covering a distance of several tens of cell diameters over the time course of an experiment, and having a polarized morphology (clearly distinct lamellipodium, cell body, and uropodium).
- Tethered cells, i.e. cells clearly polarized and apparently trying to move, but tethered at a single point to the slide surface.

- Cells having protrusions but not a clearly defined polarized morphology and not covering a significant distance.
- Non-moving cells, spherical, with no protrusions.

T cells from every time-lapse sequence were counted and classified into these four categories; the typical number of cells in a sequence was between 30 and 80 (see Table 1).

Motility

Since a single cell often changed its locomotory behaviour during a measurement, the fraction of the total observation time in which a cell belongs to one of the above categories was calculated. Tethered cells were considered as effectively moving, for the following reasons: T cells are moving in a non-physiological environment, which may partly explain tethering; moreover, T cells were often observed to switch from moving to being tethered and vice versa. Thus, an index called motility was obtained by adding the fractions of the total time the cell spent as moving and as tethered, for every cell. Averages were taken over all cells of analogous samples (i.e. same activation state and same gravity level). Results are expressed as mean ± SE.

Trajectories

In the sequences lasting 3 h (experiment A), we tracked T cells which moved for more than 1 h by a program written in MATLAB (The MathWorks, Natick, MA). The program was based on an image correlation algorithm (Tolić-Nørrelykke et al. 2002). The time threshold of 1 h was chosen in order to analyse only those cells which have moved for a reasonably large time and distance. To avoid taking into account the small shifts due to shape changes of the cell instead of real translational movements, two points in a trajectory were considered distinct only if their distance was greater than a typical T cell dimension (7 μm).

Persistence indexes and mean squared displacements

For every tracked cell, the persistence index and the mean squared displacement (MSD) were calculated. We defined the persistence index of a trajectory as the mean cosine of the turning angles, in analogy with the definition of the persistence length of a polymer (Doi and Edwards 1988). It is an index of the straightness of a trajectory, since it equals one for a perfectly straight trajectory and goes to zero for a completely random motion. To calculate the MSD of a cell at a time delay t, the distance between pairs of points with a time differ-

Table 1 Results of motility experiments

Experiment	Sample		Motility (%) ± SE (number of observed cells)				
			Day 1	Day 2	Day 3	Day 4	Day 5
A	Activated	1*g*	3.0 ± 3.0 (33)	20.9 ± 6.6 (31)	37.1 ± 8.4 (28)	21.6 ± 6.3 (30)	11.1 ± 5.7 (29)
		10*g*	11.4 ± 4.3 (45)	48.6 ± 8.0 (32)	18.2 ± 6.6 (29)	9.5 ± 6.6 (21)	9.8 ± 4.3 (39)
	Non-activated	1*g*	0.0 ± 0.0 (47)	4.3 ± 2.8 (33)	14.7 ± 5.0 (37)	–	–
		10*g*	4.3 ± 3.0 (37)	23.8 ± 4.9 (47)	24.4 ± 5.7 (41)	–	–
	Late-activated	1*g*	0.0 ± 0.0 (17)	11.0 ± 4.4 (29)	26.8 ± 6.9 (35)	39.9 ± 8.3 (33)	–
		10*g*	27.5 ± 8.9 (21)	32.7 ± 7.1 (34)	7.9 ± 4.7 (31)	20.9 ± 6.8 (29)	–
B	Activated	1*g*	12.5 ± 6.0 (31)	11.7 ± 4.5 (45)	–	–	–
		10*g*	19.8 ± 5.9 (45)	15.0 ± 5.5 (37)	–	–	–
	Non-activated	1*g*	6.1 ± 4.2 (33)	3.8 ± 2.0 (80)	4.1 ± 2.1 (83)	0.7 ± 0.7 (69)	0.0 ± 0.0 (58)
		10*g*	1.8 ± 1.8 (56)	8.8 ± 3.1 (69)	2.1 ± 1.6 (66)	1.6 ± 1.6 (45)	0.0 ± 0.0 (47)
C	Activated	1*g*	11.9 ± 5.3 (36)	15.1 ± 5.3 (38)	42.2 ± 6.2 (58)	27.5 ± 4.3 (77)	20.9 ± 3.8 (85)
		10*g*	20.0 ± 8.2 (25)	34.6 ± 7.4 (40)	58.5 ± 6.6 (43)	–	13.6 ± 2.7 (119)
	Non-activated	1*g*	7.7 ± 3.5 (52)	9.4 ± 2.9 (90)	7.0 ± 2.8 (73)	4.2 ± 2.2 (79)	5.0 ± 2.5 (63)
		10*g*	5.9 ± 2.6 (68)	0.0 ± 0.0 (47)	10.0 ± 3.7 (53)	0.0 ± 0.0 (49)	3.5 ± 2.0 (84)
D	Activated	1*g*	5.2 ± 2.3 (77)	11.2 ± 3.6 (67)	13.2 ± 3.8 (72)	18.6 ± 4.3 (65)	0.2 ± 0.2 (96)
		10*g*	10.5 ± 3.1 (90)	11.6 ± 3.4 (58)	14.2 ± 4.4 (53)	10.2 ± 2.2 (144)	1.5 ± 1.0 (151)

Summary of the motility experiments results. In each experiment (A, B, C, and D), all samples were obtained from the same buffy coat (different from experiment to experiment). The cells were activated immediately before the 10*g* centrifugation and washed before seeding on microscope slides. Experiment A included activated, non-activated, and late-activated samples (see the text). Experiments B and C had activated and non-activated cells. Experiment D had only activated cells. For every sample, the motility is shown; the number of cells over which it was calculated is in brackets. The motility of experiment C, day 4, was discarded because the cells on the slide showed a very high mortality, whereas they were viable the days before and after

ence t was squared and averaged over the trajectory. Persistence indexes and MSDs for cells pertaining to analogous samples (i.e. same activation state and same gravity level) were then averaged.

Statistics

Comparisons between two samples were performed using a Student's t test. The difference between two samples was considered statistically significant at a 5% significance level ($P < 0.05$).

Results

We investigated the effect of hypergravity (10*g*) culture on sets of T cells with different activation states in four main experiments, labelled A, B, C, and D. In each experiment, all T cells were obtained from the same buffy coat. The results of these experiments are summarized in Table 1. With regard to the activation state of the cells, we refer to:

- Activated samples, which were activated immediately after purification.
- Non-activated samples, which were not activated at all or were not activated immediately.
- Late-activated samples, i.e. the non-activated samples which were activated 7 days after separation and purification.

Motility measurements were performed on days 1–5 following separation and purification for activated and non-activated cells, and on days 1–4 following activation (corresponding to days 8–11 after separation and purification) for late-activated cells. Auxiliary experiments were performed to measure cell number, viability, and proliferation (see below).

The calculated MSDs (data not shown) and persistence indexes (values ~0.5 for all samples) indicated that, at the time scale of our experiments, the motion of T cells could not be fitted with a persistent random walk model. Therefore, we decided to describe the degree of motility with a model-independent parameter (the motility index, see Materials and methods).

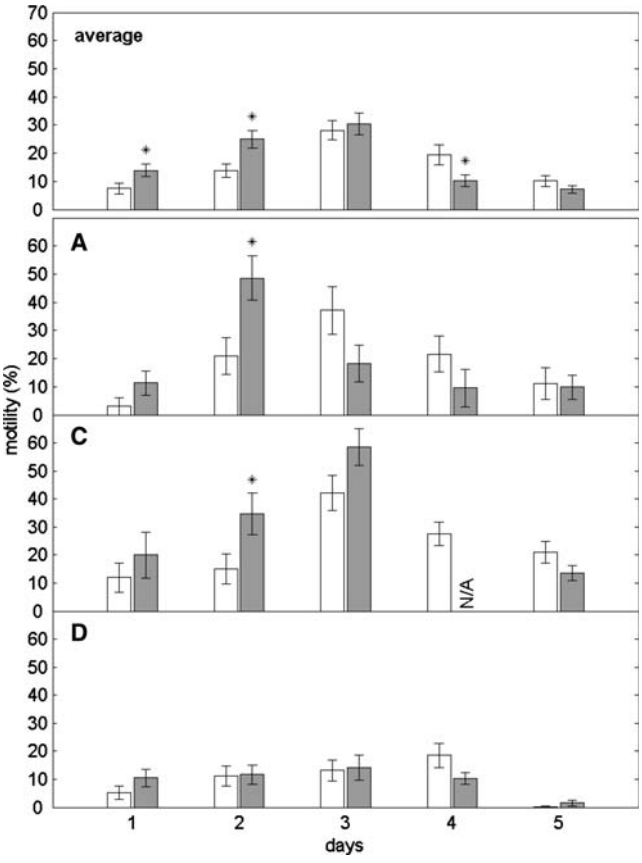

Fig. 1 Hypergravity speeds up the motility development of activated T cells. **a**, **c**, **d** motilities for activated cells of experiments A, C, and D, respectively. Experiment B is not plotted since measurements were performed only on days 1 and 2 (Table 1). *Top* average motility for the four experiments; motilities of experiment C, day 4, are not included in the average since the 10 g value is missing (see Table 1). The *horizontal axis* shows the number of days elapsed since activation and the start of the 10*g* centrifugation. *White bars* 1 g samples; *grey bars* 10 g samples; *error bars* SE. The *asterisk* denotes statistically significant difference

Motility of activated T cells peaks faster in hypergravity than in normal gravity

Figure 1 shows the motility for activated cells cultured at 1*g* and cultured at 10*g* in experiments A, C, and D (experiment B is not shown since the data were acquired only on days 1 and 2, see Table 1). The average motility of the four experiments, both 1 and 10*g* (Fig. 1, top), first increased, then reached a peak, and subsequently decreased. The measured motility maxima were found on day 3 for both 1 and 10*g* samples and were comparable, but the 10*g* motility was significantly higher than 1*g* on days 1 and 2, whereas it was smaller on day 4. Thus, the real maximum for the 10*g* sample was shifted towards earlier times compared to 1*g*: the real peak for the 1*g* sample was most likely between days 3 and 4, while it was between days 2 and 3 for the 10*g* sample.

Motility of non-activated T cells

The motility of non-activated T cells (experiments A, B, and C) did not show a reproducible behaviour between the various experiments, most likely because of the small motility values (Fig. 2). Only in experiment A, the 10*g* motility peaked faster than the 1*g* motility, as for the activated samples. In experiments B and C, the trend was not clear. The average motility therefore, although having the same trend as in the case of activated cells, did not show statistically significant differences between 1 and 10*g* cells (Fig. 2, top). Both 1 and 10*g* data in-

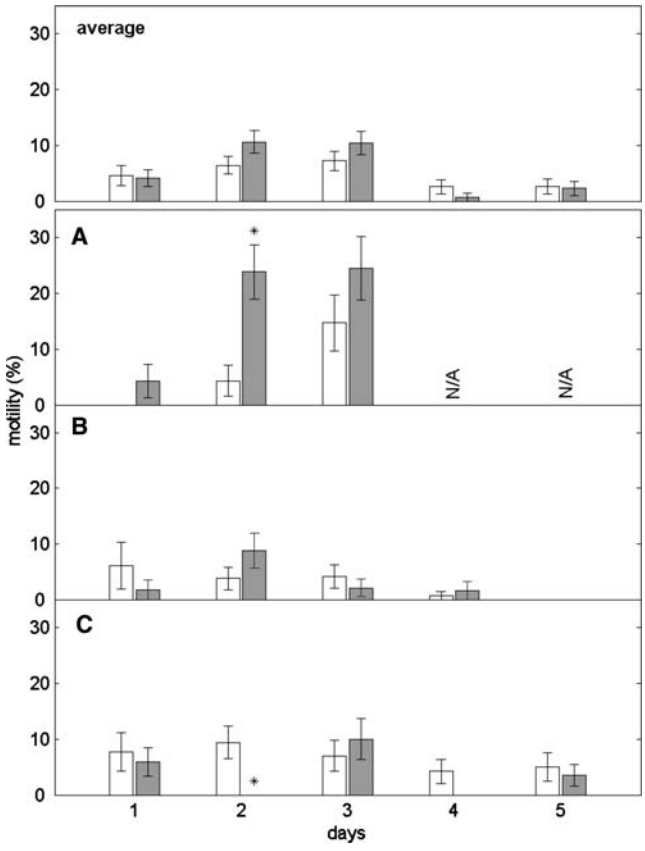

Fig. 2 Motility for non-activated T cells. **a**, **b**, **c** motilities for non-activated cells of experiments A, B, and C, respectively (Table 1). *Top* average motility for the three experiments; The *horizontal axis* shows the number of days elapsed since activation and the start of the 10*g* centrifugation. *White bars* 1 g samples; *grey bars* 10 g samples; *error bars* SE. The *asterisk* denotes statistically significant difference. The vertical scale is doubled with respect to Fig. 1 since the motility values for non-activated cells are smaller

creased from day 1 to day 3 and then decreased (as for activated cells). The overall 10*g* motility was higher than 1*g* motility, though not significantly.

The late-activated samples (experiment A only, see Table 1) had overall motility values comparable to the activated ones, showing that the culture at 10*g* before activation did not affect the development of motility.

T cell number, viability, and proliferation at 10 and 1*g*

An additional experiment (experiment E) was performed in order to count cells and measure viability and proliferation at 10*g* versus 1*g*. T cells were separated and purified from the same buffy coat, and four samples were prepared as before: activated 1*g*, activated 10*g*, non-activated 1*g*, and non-activated 10*g*. Hypergravity culture started immediately after activation. Measurements were taken after 3 and 6 days. Proliferation was considered only for activated samples.

T cell viability was high ($>80\%$) in all samples (both activated and non-activated) on days 3 and 6, and no significant difference was observed between 1 and 10*g* samples. Proliferation for activated cells decreased in time but again there was no significant difference between 1 and 10*g* cells. It is to be noted that for the 10*g* cells, the assay we used measured proliferation after the sample had been brought again to normal gravity, i.e. we measured the recovery of proliferation after 3 and 6 days of culture at 10*g*. This follows the same method of the motility experiments, where motility is measured in normal gravity after culture in hypergravity.

On the other hand, a significant difference was observed on day 6 between the 1 and 10*g* counts, when the activated 1*g* sample had a number of cells/ml almost two times higher than its 10*g* counterpart. Cells were counted also in experiment A, giving a result consistent with experiment E (data not shown).

Cell packing

A possible explanation for the observed higher growth rate of the cells at 1*g* than at 10*g* could be the clustering of cells in the flasks. Namely, the centrifugal force pushed the cells into two corners of the flasks kept in centrifuge at 10*g*. The 1*g* samples were kept tilted at a small angle to let the cells accumulate along the flask edge instead of spreading over the bottom surface, but the cell packing was less evident than in the centrifuge. The formation of large clusters of cells at 10*g* may have influenced the cell proliferation rate and/or the process of cell counting in the haemocytometer. Namely, it is possible that in the haemocytometer the clustered cells were not dispersed sufficiently, resulting in an underestimate of the cell number. The hypothesis is supported by the fact that the 10*g* cells were viable and that, immediately after stopping the centrifugation, they proliferated like the 1*g* cells (see above).

To test if the observed earlier development of motility at 10*g* could be due to the packing of cells in the flask edges during centrifugation (see Materials and methods) and not due to hypergravity itself, we performed the following experiment (experiment F): four samples of activated cells were prepared from the same buffy coat, as described above. Two of them were cultured as before in flasks (see Materials and methods), one at 1*g* and another at 10*g*. The other two samples were kept in tubes with a V-shaped bottom (Bibby Sterilin, Staffordshire, UK) at 1 and 14*g*, respectively. (We used the same homemade centrifuge for both flasks and V-bottomed tubes; since tubes are longer than flasks, the *g*-value at their bottom is slightly higher.) We assumed that the cells would accumulate at the bottom of these tubes almost independently of the gravity level. Centrifugation started immediately after activation. Motility was measured in days 2, 3, and 4—the motility for the samples cultured in tubes followed the same behaviour observed in experiments A–D: the motility peaked faster for the 14*g* cells with respect to the 1*g* cells, regardless of the fact that also at 1*g* the cells were packed. We conclude that the observed differences in motility between the 1*g* and the

10*g* activated samples of experiments A–D are due to hypergravity and not due to the packing of cells.

Discussion

Model description of T cell migration

The first step of this study was to determine migration parameters such as persistence indexes and MSDs, in order to compare the data with previous studies on lymphocyte migration. The model for lymphocyte motility which is widespread in the literature for either migration on a 2-D fibronectin-coated surface (Bergman and Zygourakis 1999), inside a gel (Lauffenburger et al. 1983), or in intact lymph nodes (Miller et al. 2002) is the persistent random walk. This model could not be well fitted to our trajectories: the calculated persistence indexes (values ~0.5) and MSDs (not shown) suggest that on the time scale of the experiment (3 h), the motion was not random but had a significant ballistic component. Whereas this model is well suited to other leukocytes such as granulocytes (Lauffenburger et al. 1983), limitations of its application to lymphocytes have already been pointed out in the study of Bergman and Zygourakis (1999). In the work by Miller et al. (2002), who use this model satisfactorily, the time scale was much shorter (~ 10 min) than in our case. Therefore, we decided to use a model-independent parameter (namely, the motility index) as a descriptor of the degree of cell motility. This approach is supported by the fact that the various classes of locomotory behaviours we observed on fibronectin in our 2-D assay were reported also in the more physiological environment of the lymph nodes (Miller et al. 2002). The authors believe that a model of T cell motion which covers a long range of time scales, and which could also explain the behaviour we observed at our time scale (~1 h), would greatly help to understand the mechanisms underlying lymphocyte migration in vitro and in vivo.

Hypergravity acts as a stimulus for T cell motility

The effects of an altered gravity environment upon different cell behaviours have been reviewed by Cogoli (1993). Although the influence of gravity on single cells has been studied both on earth and in space missions, the mechanism by which gravity affects cells has remained unclear, and it is still not possible to draw general conclusions. Our work was aimed at testing the hypothesis that, since microgravity culture, both true or simulated, influences lymphocyte motility (Cogoli-Greuter et al. 1996; Pellis et al. 1997; Sundaresan et al. 2002), hypergravity culture would as well affect the motility of T cells.

Video-microscopy observations in true microgravity (Cogoli-Greuter et al. 1996) show that lymphocytes in suspension at 0*g* are motile: the 0*g* mean velocities do not decrease in time as in normal gravity, suggesting that cells are not proceeding through the cell cycle. On the other hand, modelled microgravity induces a decrease in lymphocyte motility, measured as the maximum distance covered by the leading front cells in a 3-D gel assay, if the cells are not activated prior to 0*g* culture (Pellis et al. 1997; Sundaresan et al. 2002). In these latter works, cell motility was measured after cells were taken out from the bioreactor, thus under 1*g* conditions. Activated cells do not loose their migration capability, whereas microgravity-cultured non-activated cells are not able to recover motility even after a period of 72 h in normal gravity.

Our results show that hypergravity culture enhances the motility of T cells on a fibronectin substrate. We use a video-microscopy 2-D migration assay, where cells are observed for a few hours (1–3) in normal gravity immediately after hypergravity centrifugation. If the cells are activated before centrifugation, exposure to hypergravity seems to act as a locomotory stimulus: the motility develops earlier, i.e. the number of motile 10*g* cells peaks earlier with respect to the 1*g* sample (Fig. 1). On the other hand, if T cells are not activated, the motility values are smaller (as expected), and the poorer statistics does not allow us to draw a firm conclusion (Fig. 2). The most important difference we found from the result of Pellis and co-workers mentioned above is that in our case gravity affects cells even when they are activated before hypergravity culture. Reasons for this discrepancy could not only be the different effects of microgravity and hypergravity on the cells, but also the different methods for measuring motility used in these two studies. The analysis of time-lapse microscopic images of migrating lymphocytes allowed us to spot a subtle effect of gravity that could be difficult to see with other methods, such as the leading front assay.

On the other hand, the method we employed has a relatively poor statistics, since the number of observable cells in every sample is usually between 30 and 80 (see Table 1). Since the percentage of motile cells is usually well below 50% for activated cells, and below 30% for non-activated cells (Figs. 1, 2), and since only a fraction of these migrate continuously for a sufficient time to be tracked, it is very hard to get a good day-by-day statistics for trajectory-related parameters (e.g. MSD, persistence index, mean velocity). Hence, a direct comparison of our result with the previously cited observations in true microgravity by Cogoli-Greuter et al. (1996) is very difficult. However, from our data on activated cells, it seems reasonable to infer that both the 1*g* and the 10*g* cells are proceeding through the cell cycle.

Direct and indirect effects of gravity on single cells

Although it is relatively easy to grow cells in a hypergravitational field, it is difficult to distinguish the direct effect of the *g*-force on a single cell from the indirect effects induced on the whole sample. Squeezing of cells may have an impact on cell function, although the

cytoskeletal structure is strong enough to support gravitational loading: mammalian cells centrifuged at 10–100*g* do not show displacement of the nuclei (Todd 1989), and the displacement of other organelles seems even less presumable. The effect of pressure change is negligible, at least for activated T cells (Tschopp and Cogoli 1983). Cell packing, on the other hand, probably affects the cells, since cell–cell and cell–substrate contacts are changed, and food availability is diminished. Finally, a change in gravity affects convection, which may result in a reduced or enhanced transport of nutrients, wastes, and signalling molecules between cells.

Defects have been reported in signal transduction (Cogoli 1997; Cooper and Pellis 1998; Sundaresan et al. 2002), expression of adhesion molecules (Pellis et al. 1997), and cytoskeletal structure (Cogoli-Greuter et al. 1994; Cogoli 1997; Lewis et al. 1998; Lewis 2004; Cogoli-Greuter 2004) for cells exposed to altered gravity. In general, it has been found that in spaceflown cells the polymerization of actin and tubulin is altered, and there are anomalies in the morphology of microtubules, actin stress fibres and intermediate filament networks. In particular, observations made on concanavalin A-activated Jurkat cells on sounding rocket flights (Sciola et al. 1999) showed significant changes in the patching of the concanavalin A receptors and in the structure of vimentin. Discontinuity (possibly depolymerization) of vimentin filaments was observed almost immediately after the onset of microgravity. It has also been suggested that the observed ability of lymphocytes to swim in the culture medium when suspended at 0*g* in spaceflight may be partly due to such gravity-induced alterations (Cogoli-Greuter et al. 1996). Since even microtubule self-organization in vitro seems influenced by the *g*-level (Glade et al. 2002; Tabony 2004), it has been hypothesized that a cell as a whole may act as a gravisensing object (Ingber 1999).

Our work was not aimed at the observation of lymphocyte cytoskeleton in hypergravity. However, as the differences observed in motility behaviour between 1 and 10*g* cells are subtle, no such dramatic cytoskeletal changes are likely to happen in hypergravity (at least up to 10*g*). To support this hypothesis and to have a more complete picture, it would be very helpful to study cytoskeleton structure and expression of integrins and other receptors simultaneously with migration in cells cultured at different levels of hypergravity.

Conclusions

We studied the motility of peripheral blood T cells cultured in conditions of altered gravity (10*g*) by time-lapse microscopy and computer-assisted cell tracking. T cells were migrating on a fibronectin-coated glass surface. We found that hypergravity culture enhances motility: activated T cells become motile earlier at 10*g* than at 1*g*. Future studies should include motility of cells cultured in microgravity conditions, as well as the chemotactic behaviour of T cells exposed to altered gravity.

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