Gravitational Force: Triggered Stress in Cells of the Immune System

14

Oliver Ullrich and Cora S. Thiel

14.1 The "Immune Problem" in Space as a Cellular "Gravity Problem"

Cells of the immune system are exceptionally sensitive to microgravity. During the first Spacelab missions in the year 1983 the pioneering discovery from Cogoli and co-workers - that isolated human lymphocytes failed to proliferate after several days in microgravity - provided the first strong evidence of cell sensitivity to long-term reduced gravity exposure (Cogoli et al. 1984). Follow-up experiments clearly verified the depression of lymphocyte proliferation activation after mitogenic stimulation in long-term microgravity (Cogoli 1996).

Immunological problems of spaceflight were already described since the first Apollo missions, when more than half of the astronauts suffered from bacterial or viral infections (Hawkins and Zieglschmid 1975). Also in crew members of Skylab and Soyuz, a reduced reactivity of blood lymphoid cells has been observed (Konstantinova et al. 1973; Kimzey 1977), whereas recent studies described a reactivation of the varicella zoster virus (VZV), a latent nervous system virus, in astronauts (Cohrs et al. 2008; Mehta et al. 2004), an alarming observation for long-term space missions. Because of the obvious and severe effects on the human immune system, serious concerns arose whether spaceflight-associated immune system weakening ultimately precludes the expansion of human presence beyond Earth's

O. Ullrich (✉)
Institute of Anatomy, Faculty of Medicine, University of Zurich, Zurich, Switzerland

Department of Machine Design, Engineering Design and Product Development, Institute of Mechanical Engineering, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany
e-mail: oliver.ullrich@anatom.uzh.ch, oliver.ullrich@ovgu.de

C.S. Thiel
Institute of Anatomy, Faculty of Medicine, University of Zurich, Zurich, Switzerland

Institute of Medical Physics and Biophysics, University of Muenster, Muenster, Germany

A. Choukèr (ed.), *Stress Challenges and Immunity in Space*,
DOI 10.1007/978-3-642-22272-6_14,

orbit (Guéguinou et al. 2009) (see also Chaps 9-12, 15-16). Therefore, to understand the cellular and molecular mechanisms of how gravity changes can influence immune cell functions has become an urgent need. For the future of human space flight, we should know which cellular and molecular mechanisms will provide therapeutic or preventive targets for keeping the immune system of astronauts alive during long-term space missions.

The gravity field may act on a cell directly or indirectly. In the experimental systems used, direct influences of altered gravity are more pronounced in vitro, while indirect influences are more apparent in the living organisms (Tairbekov 1996). Therefore, in vitro experiments with living human immune cells in microgravity conditions, such as on board of parabolic flights, sounding rockets, satellites, or the International Space Station (ISS), are providing an ideal platform to elucidate the underlying cellular and molecular mechanisms. In contrast to the logistic limitations of the International Space Station (ISS) and other space-based research platforms, parabolic flights provide frequent and repeated access to microgravity and therefore allow replication and modification of experiments within a reasonable time frame, which are not only characteristics, but rather requirements, of modern biomedical research. Thus, access to space is an instrument to elucidate long-term and functional effects of microgravity, whereas ultrashort, initial and primary effects and mechanisms are amenable by short-term-microgravity provided by parabolic flight maneuvers. In vivo and in vitro experiments can be performed on board of an aircraft which is weightless when it is flying on a Keplarian trajectory, described as an unpropelled body in ideally frictionless space subjected to a centrally symmetric gravitational field. For extensive in vitro experiments with living cells of the human immune system, we recently developed an experimental system which allows for large-scale cell culture experiments with living mammalian cells on board of the parabolic flight aircraft Airbus A300 ZERO-G (Paulsen et al. 2010) and for small scale, but frequent experiments on board of the military fighter aircraft Northrop F-5E (Studer et al. 2010). Due to the fact that cells of the immune system are obviously influenced by altered gravity, its gravisensitive nature render these cells also an ideal biological model in the search for general gravisensitive mechanisms in mammalian cells (Fig. 14.1).

Whereas it has been supposed that the most of the cellular effects of microgravity or of simulated weightlessness by the clinostat model may be attributed to the generalized unspecific reaction of a cell to external influence (Kondrachuk and Sirenko 1996), recent findings support a more specific nature of the cellular reactions (Paulsen et al. 2010). The ability of cells to sense, to interpret, and to respond to mechanical forces are increasingly recognized as playing predominant roles in a variety of relevant cellular functions (Orr et al. 2006; Ingber 2003a) such as cell migration, growth, mitosis, and differentiation (Engler et al. 2006). The mechanisms by which the cells detect mechanical forces could lead to an idea how cells may sense gravitational forces. In the last decade, much progress has been made in understanding the response of cells to physical forces (Vogel and Sheetz 2006; Hoffman and Crocker 2009). However, a clear concept how human cells are sensing gravity is missing and research trying to identify and to understand gravity-sensitive mechanisms in human cells is still at the beginning of a long road.

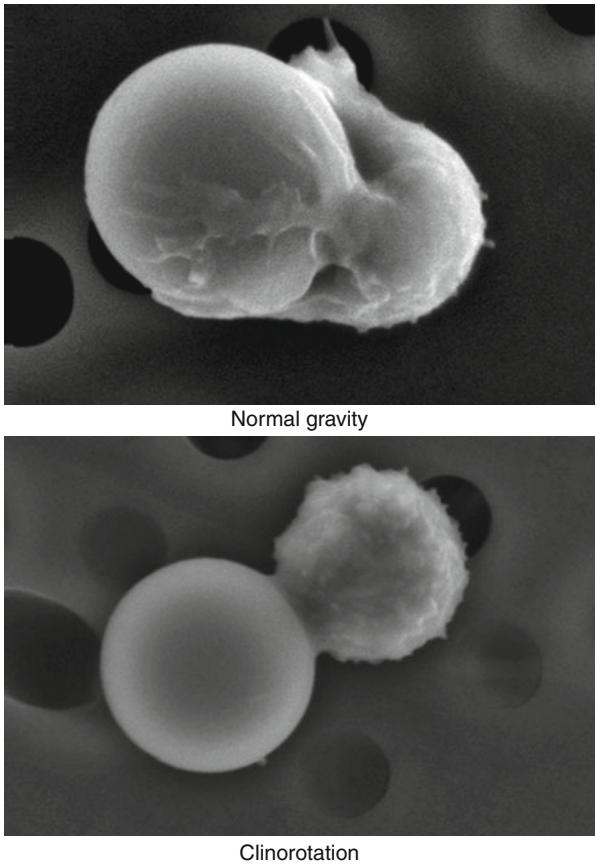

Fig. 14.1 T-cell activation in 1g (static) culture versus simulated microgravity (clinorotation); Photo credit to Clarence Sams (NASA Johnson Space Centre) and Mayra Nelman-Gonzalez (Wyle)

14.2 Gravisensitivity in Cells of the Immune System

Several investigations evidence alterations in signal transduction in lymphocytes. In this type of cells, microgravity affected the protein kinase C (Hatton et al. 2002; Schmitt et al. 1996), whereas delivery of first activation signal, patching, and capping of conA-binding membrane proteins occurred normally (Cogoli et al. 1992). These findings suggest the existence of gravisensitive cellular targets upstream from PKC (protein kinase C) and downstream from the TCR (T cell receptor)/CD3, where the lipid-raft-associated membrane-proximal signalosome complex is located. Gene expression analysis of T cells subjected to simulated microgravity revealed an alteration of several signal moduls, in particular NF-kB and MAPK-signalling (Boonyaratanakornkit et al. 2005). Also the expression of the early oncogenes c-fos, c-myc, and c-jun is inhibited (summarized in Braeucker et al. 2002). Gravisensitive mechanisms have been recently suggested at the chromatin (Paulsen et al. 2010) and epigenetic level (Singh et al. 2010). In Jurkat T cells, enhanced phosphorylation of the MAP kinases ERK-1/2, MEK, and p38 and inhibition of nuclear translocation

of NF-kB were the predominant responses to simulated weightlessness (Paulsen et al. 2010). In contrast, nonstimulated myelocytic U937 cells responded to simulated weightlessness with enhanced overall tyrosine phosphorylation and activation of c-jun, whereas PMA (12-O-Tetradecanoylphorbol-13-acetate)-stimulated U937 cells responded with reduced tyrosine phosphorylation and reduced activation of c-jun, compared with 1 g controls (Paulsen et al. 2010).

In other studies, gravisensitivity of pro- and anti-apoptotic pathways has been reported in human mononuclear cells (Bakos et al. 2001), human ML-1 thyroid-carcinoma cells (Kossmehl et al. 2002), and astrocytes (Uva et al. 2002a). On the molecular level, microgravity induced fas, p53, and bax and reduced bcl-2 (Kossmehl et al. 2002; Nakamura et al. 2003; Ohnishi et al. 1999). Interestingly, the expression of fas was elevated in Jurkat-T-cells during space flights of the shuttle missions STS (Space Transportation System)-80 and STS-95 (Cubano and Lewis 2000), suggesting an enhanced fas-fasL-mediated apoptosis of immune cells. During a 14-day space flight (SLS-2-mission) an accumulation of p53 has been found in keratinocytes and myocytes, indicating that central regulatory molecules of nuclear signal transduction and cell cycle are influenced by gravity (Ohnishi et al. 1999). In fact, p53 protein was phosphorylated in Jurkat T cells after 20 s in real microgravity (Paulsen et al. 2010). The diminished proliferative response of T cells upon stimulation during microgravity could also be caused by a reduced expression of IL-2 receptor (Schwarzenberg et al. 1999; Walther et al. 1998), resulting in an impairment of positive regulatory feedback loops. Overall, a decreased capacity of T-cells for the production of cytokines is a prominent effect of microgravity on leukocytes (Cogoli and Cogoli-Greuter 1997).

Microgravity also impaired monocyte function: During the spacelab-mission SLS-1 monocytes lost their capability of secreting IL-1 (Cogoli 1993) and of expressing IL-2-receptor (Hashemi et al. 1999). However, the molecular mechanisms are not identified. Examination of gene expression of monocytes under real microgravity demonstrated significant changes in gene induction associated with differentiation of monocytes into macrophages (Hughes-Fulford et al. 2008). Kaur et al. (2005) investigated monocytes isolated from astronauts before and after a mission and compared the results with control groups. They found a reduction of phagocytosis and a reduced oxidative burst- and degranulation-capacity. Meloni et al. (2006) recently demonstrated that simulated weightlessness leads to massive alterations in the cytoskeleton of monocytes, which in turn influences motility and recently revealed during an ISS experiment a severe reduction in the locomotion ability of monocytic cells in microgravity (Meloni et al. 2008) (see also Chap. 12). Importantly, LFA-1 (Lymphocyte function-associated antigen 1) and ICAM-1 (intercellular adhesion molecule-1) adhesion protein expression – important to tether the antigen presenting cell (APC) to the lymphocyte that is activated and that proliferates in response to the antigen presented and other (co-) activation pathways – seemed also to be sensitive to microgravity, whereas their interaction is not altered (Meloni et al. 2008). It seems that not all cell types of the immune system are as sensitive to reduced gravity: In vitro studies with natural killer cells in simulated weightlessness and in real microgravity on

board of the ISS revealed that neither cytotoxic effects nor interferon production is altered in microgravity (Buravkova et al. 2004).

14.3 Cell Migration and Cytoskeletal Architecture in Altered Gravity

Cell migration is an essential characteristic of life. Multicellular organisms must be motile to obtain nourishment, evade being eaten in their own right, respond to environmental changes, and reproduce. Likewise, unicellular organisms such as Paramecium or Loxodes must dynamically respond to fluctuations in ever-changing surroundings to assure survival. However, cell migration is also an essential characteristic of many normal and abnormal biological processes within the human organism including embryonic development, defense against infections, wound healing, and tumor metastasis (Lauffenburger and Horwitz 1996; Horwitz and Parsons 1999). Neutrophil granulocytes demonstrate the body's first line of host defense by recognizing and killing microorganisms. Neutrophil locomotion is integral for immune effector function, because the cells have to leave the blood vessels and navigate to places of infection and injury to fulfil their main task of phagocytosis. Returning astronauts of spaceflight missions exhibited a strong increase of neutrophil granulocytes immediately after landing (Kaur et al. 2004) and neutrophil chemotactic assays showed a tenfold decrease in the optimal dose–response after landing (Stowe et al. 1999). In previous studies, changes in gravity demonstrated an inhibition of lymphocyte locomotion through type I collagen (Pellis et al. 1997; Sundaresan et al. 2002), and culture of human bone marrow CD34+ cells using NASA's rotating wall vessels resulted in a decreased migration potential (Plett et al. 2004). An altered movement in microgravity was shown for leukocytes and Jurkat T cells, too (Cogoli-Greuter et al. 1996; Sciola et al. 1999), whereas the underlying signal transduction mechanisms are still illusive. On the other side, T cells become more motile after being cultured in 10 g hypergravity (Galimberti et al. 2006).

The cytoskeleton is responsible for giving a cell its shape and for generating the forces required for cell motility. It is an internal network of at least three types of cytosolic fibers: actin filaments, microtubules, and intermediate filaments. Actin, one of the most highly conserved and abundant eukaryotic proteins, is constantly polymerized and depolymerized within cells to invoke cellular motility, tissue formation, and repair (Feldner and Brandt 2002; Lee and Gotlieb 2002). Actin dynamics are considered to be the major component of the cytoskeleton responsible for cell motility. It has been shown to be essential for the migration of T lymphocytes as well as neutrophil granulocyte migration, a conclusion readily assumed as actin-depolymerizing drugs inhibit cellular motility (Hofman et al. 1999; Verschueren et al. 1995). In contrast, an intact microtubule network does not appear to be required for neutrophil migration, because microtubule-disrupting drugs such as colchicine even induce the migration of neutrophils (Niggli 2003), probably by inducing changes in the actin network.

Multiple investigators have reported that this complex network of fibers is sensitive to environmental factors such as microgravity and altered gravitational forces (Schatten et al. 2001). Several studies demonstrate modifications of the actin and microtubule cytoskeleton in microgravity. Already a few minutes of weightlessness affected the cytoskeleton of lymphocytes, astrocytes, neurons, and glial cells, disorganizing microtubules, intermediate filaments, and microfilaments (Uva et al. 2002b, 2005; Roesner et al. 2006). Morphological differences of both the microtubule and actin components of the cytoskeleton have been observed in cells grown in real and simulated microgravity (Uva et al. 2002b; Lewis et al. 1998). Hughes-Fulford (2003) reported that actin reorganization responded to the gravity level.

14.4 Learning About Graviperception in Unicellular Systems

Unicellular systems were and are frequently used as model systems to analyze and understand the influence of gravitational forces on the cellular level. Common subjects of study are ciliates like *Paramecium* and *Loxodes*, and flagellates like the algae *Euglena* (reviewed in Hemmersbach and Braeucker 2002; Haeder et al. 2005). Ciliates and flagellates are particularly interesting, because they can show positive and negative gravitaxis (movement in the same or opposite direction as the gravity vector) and gravikinesis (altered swimming velocity) due to their swimming properties and have the advantage of convenient experimental handling and observation (Planel et al. 1981, 1982; Machemer et al. 1991; Haeder et al. 1996; Hemmersbach et al. 1998; Hemmersbach and Haeder 1999). In these model organisms two different mechanisms of graviperception evolved: (1) in *Loxodes* gravity is sensed by specific statocyst-like organelles filled with barium sulfate (Müller vesicles) (Penard 1917; Rieder 1977; Fenchel and Finlay 1986); (2) in *Euglena* and *Paramecium* density differences between cytoplasm and extracellular medium activate mechanosensitive ion channels (Hemmersbach et al. 1998, 1999; Lebert and Haeder 1996; Lebert et al. 1997). This induces different signal transduction cascades where calcium, cAMP, calmodulin, and phosphorylation processes play an important role (Hemmersbach and Haeder 1999; Haeder et al. 2005; Streb et al. 2002). The sensitivity for gravity differs in *Paramecium* (0.35 g; Hemmersbach et al. 1996, 1998), *Euglena* (0.16 g and 0.12 g; Haeder et al. 1996, 1997), and *Loxodes* (<0.15 g; Hemmersbach et al. 1998). This was shown by using a slow rotating centrifuge microscope (NIZEMI) in microgravity and identifying the acceleration threshold inducing graviresponse. Below these thresholds, protists were unable to sense gravity and lost their typical gravity-based directed movements (Hemmersbach-Krause et al. 1993). These results were independent of the previous exposure to microgravity up to 12 days, although cells underwent several division cycles. Besides the described effects on gravity-based orientation, other effects can be observed due to microgravity in protists like *Paramecium*, e.g. an increased cell growth rate, increase in cell volume, decrease in total cell protein content, and lower cell calcium content (Planel et al. 1981, 1982; Planel 2004). The research on effects of microgravity on microorganisms will be of common future interest, since they represent an essential component of biological life support systems during long-term spaceflights.

14.5 The Question of Sensing Gravitational Forces in Human Cells

The gravitational force acting on a cell or a subcellular or molecular structure is equal to the mass of the structure and the acceleration toward the center of Earth. Despite the multitude of observed gravity-dependent effects on the cellular level, there is no clear idea how human cells may sense gravity. In general, direct gravisensing (e.g., by specialized cells as parts of a gravisensing organ) and indirect gravisensing, in which cells without specialized gravity detectors are affected by the acceleration, have to be distinguished (Albrecht-Buehler 1991). It is also important to mention, to which extent the direction and the amplitude of gravity is sensed. According to basic laws of physics, the weight of a single normal-sized cell seems too small compared with other cellular forces to allow them the distinction between up and down (Albrecht-Buehler 1991). In this context, thermal and mechanical noise within cells seem too high in relation to the change of force due to the change from normogravity to microgravity (Klopp et al. 2002). But since the weight of the surrounding environment is much larger, cells may be able to sense certain environmental changes caused by gravity and thus may sense indirectly at least the amplitude of gravitational forces (Albrecht-Buehler 1991).

14.5.1 Sensing Stress as Changes in the Gravitational Environment

The environment, which is sensed locally (Choquet et al. 1997), consists of cells and of the intercellular matrix. In microgravity, force-induced breakage of cell–cell- or cell–matrix-adhesion sites could be reduced. The mechanical properties of the matrix have predominant effects on different cell functions (Discher et al. 2005). The forces sufficient to activate such kind of cellular response are very low (10pN for 1 s, Jiang et al. 2006). Adhesion-mediated signalling provides cells with information about multiple parameters of their microenvironment, including mechanical characteristics (Bershadsky et al. 2006). During the last decade, several molecules and mechanisms of adhesion-mediated signalling have been identified, mostly in endothelial cells, where the response to fluid shear stress alters and regulates several cellular functions (Engler et al. 2006). In endothelial cells, integrins are crucially involved in endothelial mechanosensing of shear stress (Shyy and Chien 2002). Immunoglobulin family adhesion receptor PECAM-1 directly transmits mechanical force and, in cooperation with VE-cadherin and VEGFR-2, mediates the response of confluent endothelial cells to shear flow (Tzima et al. 2005). Interestingly, modulation of the expression of surface adhesion molecules such as ICAM-1 has been reported as the consequence of long-term microgravity (Buravkova et al. 2005; Romanov et al. 2001). Additionally, early molecular mechanisms responsible for gravity sensing of endothelial cells involve caveolae and Caveolin-1 phosphorylation (Spisni et al. 2006). Importantly, the appropriate formation and function of the immunological synapse between T cells and antigen-presenting cells require a well-defined spatial orientation of membrane adhesion molecules ICAM-1 and LFA-1 (Mossman et al. 2005). Therefore it is possible that integrin-mediated force transduction renders the immunological synapse a gravisensitive site.

14.5.2 Cellular Mechanosensory System

If the cells are able to sense gravitational forces by adhesion to the extracellular matrix, the question about the responsible mechanosensory system arises. Paradigms of cellular mechanosensing have been reviewed by Orr et al. (2006). One possibility is that the entire actin network serves as a mechanosensor. The folding state of cytoskeleton-associated proteins, which creates or masks binding sites for other proteins, depends on the strains in the actin network (reviewed in Vogel and Sheetz 2006). Forces applied to or taken from the actin network could be therefore transduced in altered binding of signal proteins to the cytoskeleton or the gain or loss of enzyme function. Consequently, microgravity may reduce the force inside the actin network, which could be then transduced into a certain biochemical signal by cytoskeleton-associated proteins. According to the tensegrity model (Fuller 1961), the whole cell is a prestressed structure (Ingber 1993, 2003b), with tensions generated by the actin–myosin network, by cellular force through focal adhesions (Choquet et al. 1997; Tamada et al. 2004), by cell–cell adhesions and polymerization of cytoskeletal elements (Wang et al. 2001, 2002; Wang and Stamenovic 2000; Stamenovic et al. 2002). A cell would not maintain its shape stability under load without a preexisting stress or prestress in the mechanical elements (Ingber 2003b). During gravitational unloading of the cell, intracellular forces induced by the prestress could be altered and transduced into a biochemical response. Force-induced changes of protein conformation and exposure of cryptic binding sites for signal proteins have been described as a possible method of mechanotransduction (reviewed in Vogel and Sheetz 2006). Unfolding of proteins with tandem-repeat domains can occur with applied forces on the order of 50–200 pN, as demonstrated by single-molecule experiments with actinin (Rief et al. 1999), filamin (Furuike et al. 2001), and spectrin (Rief et al. 1999). Tandem-repeat sequences are found in most extracellular matrix (ECM) proteins and many proteins that link the integrins to the cytoskeleton. Therefore, cells could hypothetically "measure" strain by integrating the number of unfolded domains (Hoffman et al. 2007). Many molecules are stabilized by disulphide bonds and their redox state can be therefore sensitive to force (Vogel and Sheetz 2006). In cross-linked molecules, only a small alteration in force could readily cause extensive conformational changes, suggesting binding partners of cross-linking proteins as potential signal proteins in mechanotransduction, such as including heat shock proteins, protein kinase C, Ral A (ras-related protein A), PIP2 (Phosphatidylinositol 4,5–bisphosphate), PIP 3 (Phosphatidylinositol (3,4,5)–trisphosphate), PI3-kinase (Phosphatidylinositol 3–kinase), MEKK1 mitogen-activated protein kinase kinase 1 (as reviewed in Otey and Carpen 2004; Stossel et al. 2001), and regulatory proteins for the Rho GTPases (Mammoto et al. 2007; Ohta et al. 2006). Interestingly, Rho kinase has been found to regulate the intracellular micromechanical response of adherent cells (Kole et al. 2004) and small G proteins are discussed having a significant role in mechanotransduction (Burridge and Wennerberg 2004). A special class of mechanosensing mechanisms (Orr et al. 2006) is represented by myosins. In most myosin classes, mechanical load alters ADP

release. Myosin mechanosensing is best demonstrated by myoIC during adaptation in hearing (LeMasurier and Gillespie 2005). Enzymatic activity can be regulated in response to mechanical force by opening of enzymatic cleavage sites. Fibronectin, for example, has a partially cryptic disulphide isomerase (Langenbach and Sottile 1999) and a cryptic metalloprotease activity (Schnepel and Tschesche 2000), but it is not known whether these activities can be regulated by force (Vogel and Sheetz 2006). It has been demonstrated that application of a force to fibronectin binding induced rapid local src activation forming a directional wave propagated away from the stimulation site along the plasma membrane (Wang et al. 2005). Structural motifs that could change conformation over a range of mechanical forces and could therefore exhibit mechanosensory functions are diverse (Bershadsky et al. 2006; Martinac 2004; Vogel and Sheetz 2006). Considering the myriad of multidomain cross-linking proteins in different cells and subcellular localizations, it is likely that mechanoresponse is a highly specific and specialized process and not a general and nonspecific phenomenon.

14.5.3 Force-Sensitive Ion Channels

Mechanosensitive ion channels respond to mechanical stimuli with a change in their conductive state. Either the mechanosensitive channel senses directly alterations of the lipid bilayer, or transmit forces of the cytoskeleton or the extracellular matrix via a physical connection. Ion-channel involvement in mechanosensing is well described in prokaryotic systems (Martinac 2004). However, prokaryotic ion channels are relatively force-insensitive and only open at high tensions that are approaching the lytic tensions for the lipid bilayer (Vogel and Sheetz 2006), whereas typical membrane tensions in animal cells are a thousand fold lower than the activating bacterial tensions (Sheetz 2001). Membrane channels, for example, the PKD2 Ca^{2+} channel and inner rectifier K^{+} channel in eukaryotes, are activated upon stretching (Martinac 2004), leading to channel opening, ion flux, and most probably to the recruitment and activation of downstream signalling molecules. In this context, cellular responses to forces in bone and cartilage are probably the consequence of out-of-plane forces on channel–cytoskeleton linkages (Haut Donahue et al. 2004). Possible cellular gravisensing mechanisms and gravisensitive cellular molecules and functions are summarized in Table 14.1.

14.6 Conclusion

Taken together, cellular gravisensing may not result from a direct activation of a single gravisensing molecule. Instead, gravitational stress and forces may be sensed by an individual cell in the context of altered extracellular matrix mechanics, cell shape, cytoskeletal organization, or internal prestress in the cell–tissue matrix (Ingber 1999).

Table 14.1 Cells of the immune system are exceptionally sensitive to microgravity

Possible cellular gravisensing systems
Actin network/folding state of cytoskeleton- associated proteins
Prestressed structure of the cell (tensegrity model)
Force-induced changes of protein conformation
Force-sensitive ion channels
Gravisensitive cellular molecules and mechanisms
Protein kinase C
NF-kB
MAPK-signalling
c-fos, c-myc, and c-jun
Chromatin and epigenetic level
Pro- and anti-apoptotic pathways
Cell cycle
IL-1 secretion
IL-2-receptor expression
Phagocytosis
Oxidative burst- and degranulation-capacity
Cytoskeleton
Locomotion ability
LFA-1
ICAM-1

Gravitational forces may be sensed by individual cells in the context of altered extracellular matrix mechanics, cytoskeletal organization, or internal prestress in the cell–tissue matrix and transduced into specific molecular alterations which in turn contributes to a complex disturbance of immune cell reactions and interactions

The development of cellular mechanosensitivity and mechanosensitive signal transduction was probably an evolutionary requirement to enable our cells to sense their extracellular matrix and their individual microenvironment. However, mechanosensitive mechanisms were designed to work under the condition of 1 g, but never had the possibility to adapt and adjust their reaction to conditions below 1 g. Therefore it is possible that the same mechanisms, which enable human cells to sense and to cope with mechanical stress, are potentially dangerous in microgravity. It is a major challenge to find out if our cellular machinery is able to live and to work without gravity force or if our cellular architecture will keep us dependent on the gravity field of Earth. With the completion and utilization of the International Space Station and with mission plans to Moon and Mars during the first half of our century, astronautics has entered the era of long-term space missions. Such long-term missions represent a challenge never experienced before: Small or even marginal medical problems could easily evolve to substantial challenges, which could possibly endanger the entire mission. Since crew performance is the crucial factor during space missions and since evacuation or exchange of the crew is impossible during interplanetary flights, to elucidate the underlying mechanism of limiting factors for human

health and performance in microgravity, such as for the immune system, and to identify and test potential counteractive interventions is an urgent need. Therefore, identification of gravisensitive cellular reactions will also help to understand the molecular mechanisms of disturbed immune cell function in space in order to identify, to test, and to provide new targets for therapeutic or preventive intervention related to the immune system of astronauts during long-term space missions.

Acknowledgment We gratefully acknowledge the support by the German Aerospace Center (DLR), Space Agency (grant no. 50WB0613 and no. 50WB0912) and ESA (ESTEC Contract nr 20562/07/NL/VJ ESA-CORA-GBF-2005-005). We also gratefully thank our collaboration partners DLR, ESA, EADS Astrium Space Transportation, NOVESPACE, the Swiss Air Force and the Deutsche Lufthansa.

References

Albrecht-Buehler G (1991) Possible mechanisms of indirect gravity sensing by cells. ASGSB Bull 4:25–34

Bakos A, Varkonyi A, Minarovits J, Batkai L (2001) Effect of simulated microgravity on human lymphocytes. J Gravit Physiol 8:69–70

Bershadsky A, Kozlov M, Geiger B (2006) Adhesion-mediated mechanosensitivity: a time to experiment, and a time to theorize. Curr Opin Cell Biol 18:472–481

Boonyaratanakornkit JB, Cogoli A, Li CF, Schopper T, Pippia P, Galleri G, Meloni MA, Hughes-Fulford M (2005) Key gravity-sensitive signaling pathways drive T cell activation. FASEB J 19:2020–2022

Braeucker R, Cogoli A, Hemmersbach R (2002) Graviperception and graviresponse at the cellular level. In: Horneck G, Baumstark-Khan C (eds) Astrobiology the quest for the conditions of life. Springer, Berlin/Heidelberg/New York, pp 287–333

Buravkova LB, Rykova MP, Grigorieva V, Antropova EN (2004) Cell interactions in microgravity: cytotoxic effects of natural killer cells in vitro. J Gravit Physiol 11:177–180

Buravkova L, Romanov Y, Rykova M, Grigorieva O, Merzlikina N (2005) Cell-to-cell interactions in changed gravity: ground-based and flight experiments. Acta Astronaut 57:67–74

Burridge K, Wennerberg K (2004) Rho and Rac take center stage. Cell 116:167–179

Choquet D, Felsenfeld DP, Sheetz MP (1997) Extracellular matrix rigidity causes strengthening of integrin–cytoskeleton linkages. Cell 88:39–48

Cogoli A (1993) The effect of hypogravity and hypergravity on cells of the immune system. J Leukoc Biol 54:259–268

Cogoli A (1996) Gravitational physiology of human immune cells: a review of in vivo, ex vivo and in vitro studies. J Gravit Physiol 3:1–9

Cogoli A, Cogoli-Greuter M (1997) Activation and proliferation of lymphocytes and other mammalian cells in microgravity. Adv Space Biol Med 6:33–79

Cogoli A, Tschopp A, Fuchs-Bislin P (1984) Cell sensitivity to gravity. Science 225:228–230

Cogoli M, Bechler B, Cogoli A, Arena N, Barni S, Pippia P, Sechi G, Valora N, Monti R (1992) Lymphocytes on sounding rockets. Adv Space Res 12:141–144

Cogoli-Greuter M, Meloni MA, Sciola L, Spano A, Pippia P, Monaco G, Cogoli A (1996) Movements and interactions of leukocytes in microgravity. J Biotechnol 47:279–287

Cohrs RJ, Mehta SK, Schmid DS, Gilden DH, Pierson DL (2008) Asymptomatic reactivation and shed of infectious varicella zoster virus in astronauts. J Med Virol 80:1116–1122

Cubano LA, Lewis ML (2000) Fas/APO-1 protein is increased in spaceflown lymphocytes (Jurkat). Exp Gerontol 35:389–400

Discher DE, Janmey P, Wang YL (2005) Tissue cells feel and respond to the stiffness of their substrate. Science 310:1139–1143
Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. Cell 126:677–689
Feldner JC, Brandt BH (2002) Cancer cell motility–on the road from c-erbB-2 receptor steered signaling to actin reorganization. Exp Cell Res 272:93–108
Fenchel T, Finlay BJ (1986) The structure and function of Müller vesicles in loxodid ciliates. J Protozool 33:69–76
Fuller B (1961) Tensegrity. Portfolio Artnews Annu 4:112–127
Furuike S, Ito T, Yamazaki M (2001) Mechanical unfolding of single filamin A (ABP-280) molecules detected by atomic force microscopy. FEBS Lett 498:72–75
Galimberti M, Tolic-Norrelykke IM, Favillini R, Mercatelli R, Annunziato F, Cosmi L, Liotta F, Santarlasci V, Maggi E, Pavone FS (2006) Hypergravity speeds up the development of T-lymphocyte motility. Eur Biophys J 35:393–400
Guéguinou N, Huin-Schohn C, Bascove M, Bueb JL, Tschirhart E, Legrand-Frossi C, Frippiat JP (2009) Could spaceflight-associated immune system weakening preclude the expansion of human presence beyond earth's orbit? J Leukoc Biol 86:1027–1038
Haeder D-P, Rosum A, Schaefer J, Hemmersbach R (1996) Graviperception in the flagellate Euglena gracilis during a shuttle spaceflight. J Biotechnol 47:261–269
Haeder D-P, Porst M, Tahedl H, Richter P, Lebert M (1997) Gravitactic orientation in the flagellate Euglena gracilis. Microgravity Sci Technol 10:53–57
Haeder D-P, Hemmersbach R, Lebert M (2005) Gravity and the behaviour of unicellular organisms. Cambridge University Press, Cambridge
Hashemi BB, Penkala JE, Vens C, Huls H, Cubbage M, Sams CF (1999) T cell activation responses are differentially regulated during clinorotation and in spaceflight. FASEB J 13:2071–2082
Hatton JP, Gaubert F, Cazenave JP, Schmitt D (2002) Microgravity modifies protein kinase C isoform translocation in the human monocytic cell line U937 and human peripheral blood T-cells. J Cell Biochem 87:39–50
Haut Donahue TL, Genetos DC, Jacobs CR, Donahue HJ, Yellowley CE (2004) Annexin V disruption impairs mechanically induced calcium signaling in osteoblastic cells. Bone 35:656–663
Hawkins W, Zieglschmid J (1975) Clinical aspects of crew health. In: Johnston R, Dietlein L, Berry C (eds) Biomedical results of Apollo. NASA, Washington, DC, pp 43–81
Hemmersbach R, Braeucker R (2002) Gravity-related behaviour in ciliates and flagellates. In: Cogoli A (ed) Cell biology and biotechnology in space, advances in space biology and medicine 8. Elsevier, Amsterdam, pp 59–75
Hemmersbach R, Haeder D-P (1999) Graviresponses of certain ciliates and flagellates. FASEB J 13:S69–S75
Hemmersbach R, Voormanns R, Briegleb W, Rieder N, Haeder D-P (1996) Influence of accelerations on the spatial orientation of Loxodes and Paramecium. J Biotechnol 47:271–278
Hemmersbach R, Voormanns R, Bromeis B, Schmidt N, Rabien H, Ivanova K (1998) Comparative studies of the graviresponses of Paramecium and Loxodes. Adv Space Res 21:1285–1289
Hemmersbach R, Volkmann D, Haeder D-P (1999) Graviorientation in protists and plants. J Plant Physiol 154:1–15
Hemmersbach-Krause R, Briegleb W, Haeder DP, Vogel K, Grothe D, Meyer I (1993) Orientation of Paramecium under the conditions of microgravity. J Eukaryot Microbiol 40:439–446
Hoffman BD, Crocker JC (2009) Cell mechanics: dissecting the physical responses of cells to force. Annu Rev Biomed Eng 11:259–288
Hoffman BD, Massiera G, Crocker JC (2007) Fragility and mechanosensing in a thermalized cytoskeleton model with forced protein unfolding. Phys Rev E Stat Nonlin Soft Matter Phys 76:051906
Hofman P, d'Andrea L, Guzman E, Selva E, Le Negrate G, Far DF, Lemichez E, Boquet P, Rossi B (1999) Neutrophil F-actin and myosin but not microtubules functionally regulate transepithelial migration induced by interleukin 8 across a cultured intestinal epithelial monolayer. Eur Cytokine Netw 10:227–236

Horwitz AR, Parsons JT (1999) Cell migration–movin' on. Science 286:1102–1103
Hughes-Fulford M (2003) Function of the cytoskeleton in gravisensing during spaceflight. Adv Space Res 32:1585–1593
Hughes-Fulford M, Chang T, Li CF (2008) Effect of gravity on monocyte differentiation. Paper presented at the 10th ESA Life Sciences Symposium/29th Annual ISGP Meeting/24th Annual ASGSB Meeting/ELGRA Symposium "Life in Space for Life on Earth", 22–27 June 2008 Angers, France
Ingber DE (1993) Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. J Cell Sci 104:613–627
Ingber D (1999) How cells (might) sense microgravity. FASEB J 13:S3–S15
Ingber DE (2003a) Mechanobiology and diseases of mechanotransduction. Ann Med 35:564–577
Ingber DE (2003b) Tensegrity I: cell structure and hierarchical systems biology. J Cell Sci 116: 1157–1173
Jiang G, Huang AH, Cai Y, Tanase M, Sheetz MP (2006) Rigidity sensing at the leading edge through avb3 integrins and RPTPa. Biophys J 90:1804–1809
Kaur I, Simons ER, Castro VA, Ott CM, Pierson DL (2004) Changes in neutrophil functions in astronauts. Brain Behav Immun 18:443–450
Kaur I, Simons ER, Castro VA, Ott CM, Pierson DL (2005) Changes in monocyte functions of astronauts. Brain Behav Immun 19:547–554
Kimzey SL (1977) Hematology and immunology studies. In: Johnson RS, Dietlein LF, eds. Biomedical results from Skylab, NASA SP-377. Scientific and Technical Information Office, National Aeronautics and Space Administration; Washington, DC, pp 249–282
Klopp E, Graff D, Struckmeier J, Born M, Curtze S, Hofmann M, Jones D (2002) The osteoblast mechano-receptor, microgravity perception and thermodynamics. J Gravit Physiol 9:269–270
Kole TP, Tseng Y, Huang L, Katz JL, Wirtz D (2004) Rho kinase regulates the intracellular micromechanical response of adherent cells to rho activation. Mol Biol Cell 15:3475–3484
Kondrachuk AV, Sirenko SP (1996) The theoretical consideration of microgravity effects on a cell. Adv Space Res 17:165–168
Konstantinova IV, Antropova YN, Legenkov VI, Zazhirey VD (1973) Study of reactivity of blood lymphoid cells in crew members of the Soyuz-6, Soyuz-7 and Soyuz-8 spaceships before and after flight. Space Biol Med 7:48–55
Kossmehl P, Shakibaei M, Cogoli A, Pickenhahn H, Paul M, Grimm D (2002) Simulated microgravity induces programmed cell death in human thyroid carcinoma cells. J Gravit Physiol 9:P295–P296
Langenbach KJ, Sottile J (1999) Identification of protein-disulfide isomerase activity in fibronectin. J Biol Chem 274:7032–7038
Lauffenburger DA, Horwitz AF (1996) Cell migration: a physically integrated molecular process. Cell 84:359–369
Lebert M, Haeder D-P (1996) How Euglena tells up from down. Nature 379:590
Lebert M, Richter P, Haeder DP (1997) Signal perception and transduction of gravitaxis in the flagellate Euglena gracilis. J Plant Physiol 150:685–690
Lee JS, Gotlieb AI (2002) Microtubule-actin interactions may regulate endothelial integrity and repair. Cardiovasc Pathol 11:135–140
LeMasurier M, Gillespie PG (2005) Hair-cell mechanotransduction and cochlear amplification. Neuron 48:403–415
Lewis ML, Reynolds JL, Cubano LA, Hatton JP, Lawless BD, Piepmeier EH (1998) Spaceflight alters microtubules and increases apoptosis in human lymphocytes (Jurkat). FASEB J 12: 1007–1018
Machemer H, Machemer-Roehnisch S, Braeucker R, Takahashi K (1991) Gravikinesis in paramecium: theory and isolation of a physiological response to the natural gravity vector. J Comp Physiol A 168:1–12
Mammoto A, Huang S, Ingber DE (2007) Filamin links cell shape and cytoskeletal structure to Rho regulation by controlling accumulation of p190RhoGAP in lipid rafts. J Cell Sci 120: 456–467
Martinac B (2004) Mechanosensitive ion channels: molecules of mechanotransduction. J Cell Sci 117:2449–2460

Mehta SK, Cohrs RJ, Forghani B, Zerbe G, Gilden DH, Pierson DL (2004) Stress-induced subclinical reactivation of varicella zoster virus in astronauts. J Med Virol 72:174–179

Meloni MA, Galleri G, Pippia P, Cogoli-Greuter M (2006) Cytoskeleton changes and impaired motility of monocytes at modelled low gravity. Protoplasma 229:243–249

Meloni MA, Galleri G, Pani G, Saba A, Pippia P, Cogoli-Greuter M (2008) Effects of real microgravity aboard international space station on monocytes motility and interaction with T-lymphocytes. Paper presented at the 10th ESA Life Sciences Symposium/29th Annual ISGP Meeting/24th Annual ASGSB Meeting/ELGRA Symposium "Life in Space for Life on Earth", 22–27 June 2008 Angers, France

Mossman KD, Campi G, Groves JT, Dustin ML (2005) Altered TCR signaling from geometrically repatterned immunological synapses. Science 310:1191–1193

Nakamura H, Kumei Y, Morita S, Shimokawa H, Ohya K, Shinomiya K (2003) Antagonism between apoptotic (Bax/Bcl-2) and anti-apoptotic (IAP) signals in human osteoblastic cells under vector-averaged gravity condition. Ann N Y Acad Sci 1010:143–147

Niggli V (2003) Microtubule-disruption-induced and chemotactic-peptide-induced migration of human neutrophils: implications for differential sets of signalling pathways. J Cell Sci 116: 813–822

Ohnishi T, Takahashi A, Wang X, Ohnishi K, Ohira Y, Nagaoka S (1999) Accumulation of a tumor suppressor p53 protein in rat muscle during a space flight. Mutat Res 430:271–274

Ohta Y, Hartwig JH, Stossel TP (2006) FilGAP, a Rho- and ROCK-regulated GAP for Rac binds filamin A to control actin remodelling. Nat Cell Biol 8:803–814

Orr AW, Helmke BP, Blackman BR, Schwartz MA (2006) Mechanisms of mechanotransduction. Dev Cell 10:11–20

Otey CA, Carpen O (2004) Alpha-actinin revisited: a fresh look at an old player. Cell Motil Cytoskeleton 58:104–111

Paulsen K, Thiel C, Timm J, Schmidt PM, Huber K, Tauber S, Hemmersbach R, Seibt D, Kroll H, Grote KH, Zipp F, Schneider-Stock R, Cogoli A, Hilliger A, Engelmann F, Ullrich O (2010) Microgravity-induced alterations in signal transduction in cells of the immune system. Acta Astronaut 67(9–10):1116–1125

Pellis NR, Goodwin TJ, Risin D, McIntyre BW, Pizzini RP, Cooper D, Baker TL, Spaulding GF (1997) Changes in gravity inhibit lymphocyte locomotion through type I collagen. In Vitro Cell Dev Biol Anim 33:398–405

Penard E (1917) Le genre Loxodes. Rev Suisse Zool 25:453–489

Planel H (2004) Space and life: an introduction to space biology and medicine. CRC Press, Boca Raton

Planel H, Richoilley G, Tixador R, Templier J, Bes JC, Gasset G (1981) Space flight effects on Paramecium tetraurelia flown aboard Salyut 6 in the Cytos 1 and Cytos M experiment. Adv Space Res 1:95–101

Planel H, Tixador R, Nefedov Y, Gretchko G, Richoilley G (1982) Effect of space flight factors at the cellular level: results of the CYTOS experiment. Aviat Space Environ Med 53:370–374

Plett PA, Abonour R, Frankovitz SM, Orschell CM (2004) Impact of modeled microgravity on migration, differentiation, and cell cycle control of primitive human hematopoietic progenitor cells. Exp Hematol 32:773–781

Rieder N (1977) Die Müllerschen Körperchen von Loxodes magnus (Ciliata, Holotricha): Ihr Bau und ihre mögliche Funktion als Schwererezeptor. Verhandlungen der Deutschen Zoologischen Gesellschaft, 70. Jahresversammlung, Erlangen, Gustav Fisher Verlag, Stuttgart, pp 254

Rief M, Pascual J, Saraste M, Gaub HE (1999) Single molecule force spectroscopy of spectrin repeats: low unfolding forces in helix bundles. J Mol Biol 286:553–561

Roesner H, Wassermann T, Moeller W, Hanke W (2006) Effects of altered gravity on the actin and microtubule cytoskeleton of human SH-SY5Y neuroblastoma cells. Protoplasma 229:225–234

Romanov YA, Buravkova LB, Rikova MP, Antropova EN, Savchenko NN, Kabaeva NV (2001) Expression of cell adhesion molecules and lymphocyte-endothelium interaction under simulated hypogravity in vitro. J Gravit Physiol 8:5–8

Schatten H, Lewis ML, Chakrabarti A (2001) Spaceflight and clinorotation cause cytoskeleton and mitochondria changes and increases in apoptosis in cultured cells. Acta Astronaut 49: 399–418

Schmitt DA, Hatton JP, Emond C, Chaput D, Paris H, Levade T, Cazenave JP, Schaffar L (1996) The distribution of protein kinase C in human leukocytes is altered in microgravity. FASEB J 10:1627–1634

Schnepel J, Tschesche H (2000) The proteolytic activity of the recombinant cryptic human fibronectin type IV collagenase from E. coli expression. J Protein Chem 19:685–692

Schwarzenberg M, Pippia P, Meloni MA, Cossu G, Cogoli-Greuter M, Cogoli A (1999) Signal transduction in T lymphocytes–a comparison of the data from space, the free fall machine and the random positioning machine. Adv Space Res 24:793–800

Sciola L, Cogoli-Greuter M, Cogoli A, Spano A, Pippia P (1999) Influence of microgravity on mitogen binding and cytoskeleton in Jurkat cells. Adv Space Res 24:801–805

Sheetz MP (2001) Cell control by membrane–cytoskeleton adhesion. Nat Rev Mol Cell Biol 2:392–396

Shyy JY, Chien S (2002) Role of integrins in endothelial mechanosensing of shear stress. Circ Res 91:769–775

Singh KP, Kumari R, Dumond JW (2010) Simulated microgravity-induced epigenetic changes in human lymphocytes. J Cell Biochem 111(1):123–129

Spisni E, Toni M, Strillacci A, Galleri G, Santi S, Griffoni C, Tomasi V (2006) Caveolae and caveolae constituents in mechanosensing: effect of modeled microgravity on cultured human endothelial cells. Cell Biochem Biophys 46:155–164

Stamenovic D, Mijailovich SM, Tolic-Norrelykke IM, Chen J, Wang N (2002) Cell prestress. II: contribution of microtubules. Am J Physiol Cell Physiol 282:C617–C624

Stossel TP, Condeelis J, Cooley L, Hartwig JH, Noegel A, Schleicher M, Shapiro SS (2001) Filamins as integrators of cell mechanics and signalling. Nat Rev Mol Cell Biol 2:138–145

Stowe RP, Sams CF, Mehta SK, Kaur I, Jones ML, Feeback DL, Pierson DL (1999) Leukocyte subsets and neutrophil function after short-term spaceflight. J Leukoc Biol 65:179–186

Streb C, Richter P, Ntefidou M, Lebert M, Haeder DP (2002) Sensory transduction of gravitaxis in *Euglena gracilis*. J Plant Physiol 159:855–862

Studer M, Thiel C, Bradacs G, Engelmann F, Engeli S, Huerlimann E, Zeitner P, Ullrich O (2010) Parabolic maneuvers of the Swiss Air Force fighter jet Northrop F5-E as a new platform to identify rapid gravi-responsive mechanisms in cultured mammalian cells. Paper presented at the 61st International Astronautical Congress, IAC-10. A1.7.9, 27 Sep–01Oct 2010, Prague Czech Republic

Sundaresan A, Risin D, Pellis NR (2002) Loss of signal transduction and inhibition of lymphocyte locomotion in a ground-based model of microgravity. In Vitro Cell Dev Biol Anim 38: 118–122

Tairbekov MG (1996) The role of signal systems in cell gravisensitivity. Adv Space Res 17: 113–119

Tamada M, Sheetz MP, Sawada Y (2004) Activation of a signaling cascade by cytoskeleton stretch. Dev Cell 7:709–718

Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA (2005) A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature 437:426–431

Uva BM, Masini MA, Sturla M, Tagliafierro G, Strollo F (2002a) Microgravity-induced programmed cell death in astrocytes. J Gravit Physiol 9:P275–P276

Uva BM, Masini MA, Sturla M, Prato P, Passalacqua M, Giuliani M, Tagliafierro G, Strollo F (2002b) Clinorotation-induced weightlessness influences the cytoskeleton of glial cells in culture. Brain Res 934:132–139

Uva BM, Strollo F, Ricci F, Pastorino M, Mason JI, Masini MA (2005) Morpho-functional alterations in testicular and nervous cells submitted to modelled microgravity. J Endocrinol Invest 28:84–91

Verschueren H, van der Taelen I, Dewit J, De Braekeleer J, De Baetselier P, Aktories K, Just I (1995) Effects of *Clostridium botulinum* C2 toxin and cytochalasin D on in vitro invasiveness, motility and F-actin content of a murine T-lymphoma cell line. Eur J Cell Biol 66:335–341

Vogel V, Sheetz M (2006) Local force and geometry sensing regulate cell functions. Nat Rev Mol Cell Biol 7:265–275

Walther I, Pippia P, Meloni MA, Turrini F, Mannu F, Cogoli A (1998) Simulated microgravity inhibits the genetic expression of interleukin-2 and its receptor in mitogen-activated T lymphocytes. FEBS Lett 436:115–118

Wang N, Stamenovic D (2000) Contribution of intermediate filaments to cell stiffness, stiffening, and growth. Am J Physiol Cell Physiol 279:C188–C194

Wang N, Naruse K, Stamenović D, Fredberg JJ, Mijailovich SM, Tolić-Nørrelykke IM, Polte T, Mannix R, Ingber DE (2001) Mechanical behavior in living cells consistent with the tensegrity model. Proc Natl Acad Sci USA 98:7765–7770

Wang N, Tolić-Nørrelykke IM, Chen J, Mijailovich SM, Butler JP, Fredberg JJ, Stamenović D (2002) Cell prestress. I. Stiffness and prestress are closely associated in adherent contractile cells. Am J Physiol Cell Physiol 282:C606–C616

Wang Y, Botvinick EL, Zhao Y, Berns MW, Usami S, Tsien RY, Chien S (2005) Visualizing the mechanical activation of Src. Nature 434:1040–1045