Chapter 5
Cellular Effects of Altered Gravity on the Human Adaptive Immune System

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5.1 Introduction

During the evolution of life, as the always predominant factor, the Earth's gravitational field has shaped the architecture of all biological systems decisively. It is therefore not surprising that sudden changes in gravity lead to discrepancies in the normal functions of life and of our immune system [see Chap. 1].

According to current knowledge, residence in microgravity strongly influences the human body and leads to a variety of deconditioning symptoms such as bone demineralization, muscle atrophy, reduced performance of the cardiovascular system, altered neurovestibular perception, and a strong deterioration of the immune system (Moore et al. 1996) [see also Chap. 3]. In brief, astronauts showed immune system depression, reduced activation of T lymphocytes, and reactivation of latent

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viruses. (Kimzey 1977; Sonnenfeld and Shearer 2002; Stowe et al. 2001; Mehta et al. 2014).

However, the immune system is not only responsible to defend infections, it is also essential for wound healing, tissue reorganization, and repair. Thus, there are serious reasons to believe that astronauts are exposed to high risk by not only being more susceptible to infections, but also by having poor wound healing and tissue repair. As plans emerge for humans to embark on long-term spaceflights to Mars, Moon, and asteroids in the future, the health risks of a defective regulation of the immunity during spaceflight are important to comprehend. Therefore, the understanding of how gravity affects immune cell function is the key to maintain a proper immune system of astronauts. But which cellular and molecular structures may require gravity for proper function and are thus dependent on the gravity on Earth?

Since the pioneering discovery of Cogoli et al. during the first Spacelab-Mission in 1983, it is well known that proliferative response of lymphocytes to mitogenic stimulation is suppressed in microgravity (Cogoli et al. 1984; Cogoli 1996). Follow-up experiments performed to verify these results demonstrated clearly that factors other than microgravity can be excluded to be responsible for the depressed activation of lymphocytes. Whereas the phenomenological effect of reduced activation of T cells in microgravity is well described and verified (Grove et al. 1995), the fundamental molecular mechanisms remain to be discovered.

For more than 30 years, in vitro experiments with isolated T lymphocytes, the key cell type of the adaptive immune system, have been performed using different research platforms that provide real and simulated microgravity. These experiments have confirmed the effects of altered gravity on the cellular level. Thus, isolated lymphocytes prove to be a suitable biological model system for studying whether and how Earth's gravity is mandatory for cellular and molecular processes in mammalian cells. Numerous experiments in real microgravity of different length have been carried out during manned space missions, on board of orbital and suborbital flights (sounding rockets), and during parabolic flights. Studies using ground-based facilities with the aim to simulate the state of microgravity have supported these experiments in real microgravity. These facilities include fast-rotating clinostats, rotating wall vessel (RWV) bioreactors, random positioning machines (RPMs), and high-aspect ratio vessels (HARVs). As their results are comparable with results of experiments in real microgravity, RWVs and clinostats are recognized and valuable tools for simulating microgravity in suspension cell cultures (Herranz et al. 2013).

These studies in real and simulated microgravity were able to achieve new insights into gravity-sensitive functions in nonactivated and activated T lymphocytes, for example, cell cycle regulation (Thiel et al. 2012), epigenetic regulation (Singh et al. 2010), chromatin modification (Paulsen et al. 2010), differential gene expression (Chang et al. 2012; Thiel et al. 2012), altered microRNA expression profile (Mangala et al. 2011; Girardi et al. 2014; Hughes-Fulford et al. 2015), cell motility (Pellis et al. 1997; Sundaresan et al. 2002), and regulation of programmed cell death (Cubano and Lewis 2000; Lewis et al. 1998; Battista et al. 2012). Also the secretion of cytokines such as interleukin-2 (IL-2) and interferon-gamma (IFNγ) is influenced by gravitational changes (Hashemi et al. 1999; Chapes et al. 1992).

This chapter provides an overview of the results obtained over the last 30 years in in vitro experiments using T lymphocytes performed in space and in ground-based facilities with special emphasis on the used cell culture conditions. These results contribute to our current knowledge of how gravitational changes affect human T lymphocytes in vitro.

5.1.1 Regulation of T Lymphocytes in Real and Simulated Microgravity Experiments In Vitro

Up to now, several in vitro studies have been carried out in order to investigate effects of gravitational changes on isolated T lymphocytes. Representative studies demonstrating cellular and molecular alterations that have been observed in real and simulated microgravity are summarized in Tables 5.1 and 5.2.

5.1.2 T Cell Activation Is Diminished in Microgravity

The first experiments were carried out in terms of studying phenomenological effects of microgravity on isolated T lymphocytes. So far it is well known that microgravity influences T lymphocytes by diminishing the reactivity of T lymphocytes to mitogenic stimulation during spaceflight (Cogoli et al. 1984, 1988; Cogoli and Cogoli-Greuter 1997; Bechler et al. 1986). Experimental studies using the ground-based facilities RWV, clinostat, and RPM yielded the same results (Schwarzenberg et al. 1999; Cooper and Pellis 1998; Hashemi et al. 1999).

Next, the question arose whether binding of the mitogen Concanavalin A (ConA) to the T cell receptor might be changed due to the absence of gravity and therefore leads to reduced T cell activation. But this assumption was disproved in four different experiments on board of sounding rockets (Cogoli-Greuter et al. 1997; Sciola et al. 1999). By means of fluorescently labeled ConA, these experiments showed that binding of the mitogen on T cell receptors was in principal not affected; only a slight delay of patching and capping was observed.

5.1.3 T Cell Function Remains Unchanged Despite Reduced Cell-Cell and Cell-Substrate Interactions

Another factor that could lead to an inhibited T cell function may be the lack of sedimentation under microgravity conditions. This could lead to reduced cell-cell and cell-substrate interactions which in turn could be responsible for the reduced proliferative response to mitogenic stimuli. However, the cultivation of peripheral blood mononuclear cells (PBMCs) in Teflon bags, which show a reduced cell-substrate interaction, had no effect on the proliferation of phytohemagglutinin

Table 5.1 Summary of experiments performed under real microgravity conditions

Year	Author	Cell type	Research platform	Activation	Exposure/activation time	Gravity conditions	Results
2015	Hughes-Fulford et al.	Primary T cells	ISS	ConA/CD28	1.5 h	µg 1 g in-flight 0.5 g in-flight	Suppressed expression of the miRNA miR-21 after 1.5 h T cell activation due to altered gravity. Downregulation of 85 genes associated with T cell signaling. 17 out of the 85 genes were defined as targets of miR-21
2015	Tauber et al.	Primary CD4+ T cells	Parabolic flight	Nonstimulated	20 s	µg 1 g in-flight hyp-g	Suppressed the CD3 and IL-2R-surface receptor expression and reduced phosphorylation of the LAT protein due to hypergravity
2013	Tauber et al.	Primary CD4+ T cells	Sounding rocket	ConA/CD28	6 min	µg 1 g in-flight hyp-g 1 g GC	Dysregulation of several key signal proteins involved in early TCR signaling during the hypergravity phase. No further disturbance of these key proteins in the following microgravity phase
2012	Battista et al.	PBMCs	ISS	ConA	0, 3, 24, and 48 h	µg 1 g in-flight 1 g GC	Increased DNA fragmentation, PARP protein expression, p53 mRNA expression, and calpain mRNA expression. Early increase of 5-LOX activity
2012	Chang et al.	Primary T cells	ISS	ConA/CD28 or CD3/CD28 beads	1.5 h	µg 1 g in-flight	Inhibited transcription of immediately early genes. Disrupted activation of Rel/NF-kB, CREB1, and SRF transcription factors
2012	Thiel et al.	Jurkat cells, $CD4^+$ T lymphocytes	Parabolic flight	PMA or CD3/CD28	20 s	µg 1 g in-flight	Enhanced p21 Waf1/Cip1 mRNA expression due to microgravity being dependent from histone acetylation. Enhanced Tyr15-phosphorylation of cdc2

2010	Paulsen et al.	Jurkat cells	Parabolic flight	PMA or CD3/CD28	20 s	µg 1 g in-flight 1 g GC	Increased p53 phosphorylation in nonactivated as well as in activated cells due to microgravity. Enhanced MEK phosphorylation in activated cells in microgravity compared to 1 g in-flight
2008	Crucian et al.	Whole blood culture of astronauts	Space shuttle and ISS	CD3/CD28	Short- and long-duration space flight	Pre-flight: L-180, L-65, L-10, post-flight: R+0, R+3, R+14, R+30	Shuttle crewmembers: elevated early T cell activation after space flight but decreased percentage of T cell subsets capable of being stimulated to produce IL-2 and IFNγ. ISS crewmembers: reduced early T cell activation after space flight, reduced percentage of T cells capable of producing IL-2, but unchanged IFNγ percentages
2002	Hatton et al.	Primary T cells	Space shuttle	PDBu/ ionomycin or CD3 beads	0, 10, 60 min	µg 1 g in-flight 1 g GC 1.4 g ground centrifuge	Reduced translocation of PKCδ to particular cell fractions in microgravity. Unvarying PKCβII translocation in microgravity compared to 1 g
2001	Schatten et al.	Jurkat cells	Space flight	Nonstimulated	4, 48 h	µg 1 g in-flight 1 g GC 1.4 g ground centrifuge	Occurrence of mitochondria clustering and morphological alterations of mitochondrial cristae. Occurrence of cell divisions during space but also of apoptotic cells. Uneven distribution of mitochondria in cells

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Table 5.1 (continued)

Year	Author	Cell type	Research platform	Activation	Exposure/activation time	Gravity conditions	Results
2001	Lewis et al.	Jurkat cells	Space shuttle	Nonstimulated	4, 24, 48 h	μg 1 g GC ground vibration samples	Upregulated gene expression of 11 cytoskeleton-related genes in space. Differential expression of genes regulating growth, metabolism, signal transduction, adhesion, transcription, apoptosis, and tumor suppression
2000	Cubano and Lewis	Jurkat cells	Space shuttle	Nonstimulated	0, 4, 24, 48, and 75 h	μg 1 g in-flight 1 g GC 1.4 g ground centrifuge	Increased apoptosis during flight. Time-dependent and microgravity-related release of sFas
2000	Crucian et al.	PBMCs	Space shuttle	PMA/ ionomycin or PMA or PHA	5 h or 24 h	Pre-flight: L-10, post-flight: R+0, R+3	Reduced ability of CD4+ and the CD8+ T cell subsets to produce IL-2 following space flight. Reduced IFNγ production in the CD4+ T cell subset, and unchanged production of IFNγ in the CD8+ T cell subset
1999	Sciola et al.	Jurkat cells	Sounding rocket	ConA	12 min	μg 1 g in-flight 1 g GC	Unaffected binding of ConA to membrane by microgravity and slight retardation of patching. Structural changes of vimentin in microgravity: increasing presence of large bundles in microgravity. No changes in the structure of actin and in the co-localization of actin at the inner side of the cell membrane with ConA receptors after binding of the mitogen

1999	Hashemi et al.	PBMCs, purified T cells	Space shuttle	Okt3/CD28 beads or PDB/ ionomycin or Leu4 or Leu4 beads	24 h	µg 1 g in-flight	Suppressed CD25 and CD69 surface expression after activation
1998	Lewis et al.	Jurkat cells	Space shuttle	Nonstimulated	4, 24, 48 h	µg 1 g in-flight 1 g GC 1.4 g ground centrifuge	Structural changes in the microtubule cytoskeleton: shortened, coalesced filaments lacked normal branching at the cell membrane, and MTOCs were disrupted. Time-dependent increase in the apoptosis-related factor, Fas/ APO-1 in culture medium of flown cells (0 g + 1 g)
1997	Cogoli-Greuter et al.	PBMCs, Jurkat cells	Sounding rocket	ConA	7 min and 12.5 min	µg 1 g GC hyp-g (13 g)	Unaffected binding of ConA to membrane by microgravity and slight retardation of patching and capping. Occurrence of structural changes of intermediate filaments of vimentin as well as of the microtubule network. Occurrence of cell motility and cell-cell-contacts in microgravity
1996	Schmitt et al.	Jurkat cells	Space shuttle	A23187 calcimycin	1 h	µg 1 g in-flight 1 g GC 1.4 g ground centrifuge	G-level-dependent relative distribution of PKC in the cytosolic and nuclear fractions, with cytosolic PKC increasing with increasing g level, whereas nuclear PKC decreased

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Table 5.1 (continued)

Year	Author	Cell type	Research platform	Activation	Exposure/activation time	Gravity conditions	Results
1996	Cogoli-Greuter et al.	PBMCs	Space shuttle	ConA	46 and 78 h	μg 1 g GC	Aggregate forming of activated cells in microgravity as well as in ground control indicating that cell-cell contacts occur. No decrease of the mean velocity of free cells with increasing exposure time indicating that cell cycle progression is inhibited
1996	Pippia et al.	PBMCs	Space shuttle	ConA	72 h	μg 1 g in-flight 1 g GC 1.4 g ground centrifuge	No rescue of activability by addition of exogenous IL-1 + IL-2, but recovery of IFNγ production in microgravity
1993	Cogoli et al.	PBMCs	Space shuttle	ConA or ConA/ Cytodex beads	52 and 71 h	μg 1 g in-flight 1 g GC	Strong increase of IL-2 production. IL-2R expression was in the normal range with Cytodex beads in microgravity
1992	Bechler et al.	PBMCs	Space shuttle	ConA or ConA/ Cytodex beads	72 h	μg 1 g in-flight 1 g GC	Almost doubled activation in microgravity when bound to Cytodex beads. Increase of IFNγ production by 300 % on Cytodex beads
1992	Chapes et al.	PBMCs	Space shuttle	ConA	24 and 48 h	μg 1 g GC	Higher secretion of IFNγ in space than on the ground
1991	Limouse et al.	Jurkat cells or co-culture of Jurkat + THP-1 cells	Biosatellite	PMA/A23187 calcimycin, or anti-CD3 mAb in the presence of THP-1 cells	24 h	μg 1 g GC	Suppression of IL-2 secretion in microgravity. Occurrence of cell-to-cell contacts in microgravity, leading to normal production of IL-1 and IL-2 compared to ground controls

1985	Cogoli et al.	PBMCs	Space shuttle	ConA	36, 48, 72, and 96 h	µg 1 g in-flight	Reduced mitogenic activation of human lymphocytes by 90% in microgravity
1985	Cogoli et al.	Whole blood culture of astronauts	Space shuttle	ConA	78 h	Pre-flight: L-9, L-2. In-flight: L+3. Post-flight: R+0, R+7, R+13 1 g in-flight	Weakened activation of lymphocytes during and post-flight compared to the pre-flight values. Lack of lymphocyte activation in microgravity compared to 1 g in-flight control
1984	Cogoli et al.	PBMCs	Space shuttle	ConA	71 h	µg 1 g GC	Almost complete absence of mitogenic activation of human lymphocytes in microgravity compared to ground control

Modified from Hauschild et al. (2014)

1 g GC 1 g ground control, *5-LOX* 5-lipoxygenase, *APO* apoptosis antigen, *ConA* Concanavalin A, *FBS* fetal bovine serum, *HEPES* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, *HS* human serum, *hyp-g* hypergravity, *IL* interleukin, *IFN* interferon, *ISS* International Space Station, *PBMCs* peripheral blood mononuclear cells, *PDB* phorbol dibutyrate, *PHA* phytohemagglutinin, *PMA* phorbol-12-myristate-13-acetate, *PKC* protein kinase C, *PARP* poly (ADP-ribose) polymerase, *RPMI-1640* Roswell Park Memorial Institute-1640 medium, *STS* space transportation system, *TCR* T cell receptor, *TNF* tumor necrosis factor, *n/a* not available

Table 5.2 Summary of experiments performed in ground-based facilities in simulated microgravity

Year	Author	Cell type	Research platform	Activation	Exposure/activation time	Gravity conditions	Results
2015	Tauber et al.	Primary $CD4^+$ T cells	Clinostat	Nonstimulated or ConA/CD28	5, 15, 30, and 60 min	1 g sim. μg CC	Reduced expression of CD3 surface receptor and ZAP-70 protein, as well as increased histone H3 acetylation in activated cells after 5 minutes clinorotation. Transient downregulation of CD3 and stable downregulation of IL-2R after 60 minutes clinorotation
2014	Wei et al.	PBLs	RCCS	PHA	72 h	1 g sim. μg	Unchanged number of chromosomes and no structural changes. Enhanced expression of ATR in parallel with the inhibition of cell proliferation. Downregulation of the expression of DNA replication genes and DNA repair genes. Enhanced structural chromosome instability of human PBL cells in simulated microgravity
2014	Girardi et al.	PBLs	RWV	PHA and IL-2	24, 48, and 72 h	1 g sim. μg	Differential expression of 42 miRNAs in RWV-incubated PBLs compared with 1 g controls. miR-9-5p, miR-9-3p, miR-155-5p, miR-150-3p, and miR-378-3p were the most dysregulated correlating with genes involved in immune/inflammatory response, apoptosis, and cell proliferation
2014	Benavides Damm et al.	PBMCs, primary T cells	RPM	ConA (PBMCs) or ConA/CD28 (T cells)	20-22 h	1 g sim. μg, 0.2 g, 0.4 g, 0.6 g	Lymphocyte activation depends on partial gravity exposure. Equally poor activation of cells exposed to 0.2 g and 0 g, but still existing activation in cells exposed 0.6 g equally to the 1 g control. The activation level of cells exposed to 0.4 g was about in the middle of 0.2 g and 0.6 g

2012	Chang et al.	Primary CD4+ T cells	RWV	CD3/CD28 beads	1.5 h	1 g sim. μg	Reduced expression of the immediately early genes cREL, TNF, EGR1, EGR2, and JUNB under RWV conditions
2012	Thiel et al.	Jurkat	Clinostat	CD3/CD28 or PMA	5, 10, and 15 min	1 g sim. μg	Differential protein expression of cell cycle regulatory proteins: enhanced expression of p21 Waf1/Cip1 protein, less cdc25C protein expression, and enhanced Ser147-phosphorylation of cyclin B1 after CD3/CD28 stimulation
2011	Mangala et al.	TK6 human lymphoblastoid cells	RWV	Nonstimulated	72 h	1 g sim. μg	Altered miRNA expression influencing several genes that are involved in the regulation of the NF-kB-related pathway network
2010	Paulsen et al.	Jurkat	Clinostat	PMA or CD3/CD28	5 min	1 g sim. μg	Enhanced phosphorylation of the MAP kinases ERK-1/2, MEK, and p38 and inhibited nuclear translocation of NF-kB, either in nonstimulated or in stimulated cells
2010	Singh at al.	Human T cells	RWV	Nonstimulated	72 h and 7 days	1 g sim. μg	Microgravity-induced epigenetic changes in DNA methylation and chromatin histone modifications (decreased expression of DNMT1 and HDAC1)

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Table 5.2 (continued)

Year	Author	Cell type	Research platform	Activation	Exposure/activation time	Gravity conditions	Results
2010	Simons et al.	Primary CD4+ T cells	RWV	CD3/CD28	5-90 min	1 g sim. μg	Intact TCR signaling through DAG during PWV exposure. Thus, simulated microgravity might prevent T cell activation more likely by modulating the cellular response to the TCR signal rather than by annulling or limiting the signal itself
2009	Kumari et al.	Human T cells	RWV	Nonstimulated	4 h, 72 h, and 7 days	1 g sim. μg	Decreased expression of DNA repair genes, of cell cycle genes and of anti- and pro-apoptotic genes. Occurrence of DNA damage
2009	Martinelli et al.	PBMCs	Clinostat	PHA	24-48 h	1 g sim. μg	Decreased proliferation and viability after 48 h of rotation in the 3-D clinostat
2009	Simons et al.	PBMCs	RWV	PHA or PHA + PMA	48 h	1 g sim. μg	Recovery of PHA-induced activation of the CD8+ and CD4+ T cell subsets as well as naïve and memory CD4+ T cells due to PMA co-stimulation
2009	Sundaresan and Pellis	PBMCs	RWV	Nonstimulated	24 and 72 h	1 g sim. μg	Downregulation of T cell activation genes DAG kinase, Ser/Thr kinase, and Tyr kinase. Upregulation of HSPA1A (e.g., HSP-70) and downregulation of HSP9A9B (i.e., HSP-90). Upregulation of angiogenic factor PIGf
2006	Morrow	PBMCs	Clinostat	CD3/CD28 beads	2.5-16 h	1 g sim. μg	Continuing existence of Ca++/Cn signaling active, but inhibited PKC pathway since activation of fos and NF-kB is inhibited

2006	Ward et al.	Preliminary activated T cells	RWV	CD3/IL-2	24 h	1 g sim. μg	Changes in the expression of genes belonging to functional categories immune response, cell proliferation and differentiation, protein folding, transport and degradation, as well as apoptosis
2005	Degan et al.	PBMCs, lymphoblastoid cell lines LB and COR3	RPM	ConA	8 or 24 h	1 g sim. μg	Transient occurrence of apoptosis induction, release of sFas, and fluctuation of PARP activity. Decrease in intracellular concentration of ATP. Microgravity exposure might induce a condition of metabolic "quiescence"
2005	Boonyarata-nakornkit et al.	Primary T cells	RPM	ConA/CD28	30 min	1 g sim. μg	Upregulation of 99 genes significantly upregulated during early T cell activation under 1 g condition. No induced gene-expression of those genes in simulated microgravity. 28 % of these genes were component of NF-kB signaling or had evidence for regulation by NF-kB. Blocking of CREB activation by phosphorylation
2005	Risso et al.	Primary T cells	Clinostat	CD69/PMA	48 h	1 g sim. μg	Significant higher calcium concentration in clinorotated activated cells. Prolonged mitochondrial membrane hyperpolarization due to activation since after 20 h followed by depolarization in a fraction of cell population. Inhibition of IL-2 secretion and proliferation of activated cells

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Table 5.2 (continued)

Year	Author	Cell type	Research platform	Activation	Exposure/activation time	Gravity conditions	Results
2004	Sundaresan et al.	PBMCs	RWV	Nonstimulated	24, 48, 72, and 96 h	1 g sim. μg	Decrease expression of specific calcium-independent PKC isoforms in PBMCs at both the RNA and protein levels. 56 % decrease in phosphorylated PLC-γ1
2002	Galleri et al.	Primary T cells	RPM	ConA/CD28 + Protein G	6, 15, and 30 min	1 g sim. μg	Altered PKC isoform distribution in the three fractions nucleus, cytosol, and plasma membrane
2002	Sundaresan et al.	PBMCs	RWV	PMA	24, 48, 72, and 96 h	1 g sim. μg	Inhibition of locomotion of nonstimulated PBMCs after 24 h with extent of locomotion loss at 72 h. Restoration of locomotion by addition of PMA to the cells
2001	Risin and Pellis	PBMCs, preliminary activated primary T cells	RWV	CD3/IL-2	2, 4, 6, 18, and 24 h	1 g sim. μg	Inhibition of radiation- and activation-induced programmed cell death
1999	Licato and Grimm	PBMCs	RWV	IL-2	2-8 days	1 g sim. μg	Maintenance of cell viability by addition of exogenous IL-2 but no induction of CD25 surface expression or restoration of cytokine (IFNγ, IL-1β, and TNFγ) secretion
1999	Schwarzenberg et al.	PBMCs	RPM	ConA	72 h	1 g sim. μg	Suppressed proliferation of ConA-activated PBMCs of the same order of magnitude as in space after 72 h exposure to RPM

1999	Hashemi et al.	PBMCs, primary T cells	2D-clinostat	PHA or Leu4	3, 24, and 48 h	1 g sim. μg	Reduced proliferation of PHA or Leu4-stimulated PBMCs after 48 h clinorotation. Suppressed expression of CD69 and CD25 activation marker in Leu4 stimulated PBMCs after 24 h clinorotation. Inhibition of cell cycle progression. Slower TCR internalization in simulated microgravity. Restoration of surface CD69 and CD25 expression by PMA co-stimulation of activated PBMCs
1998	Walther et al.	PBMCs	Clinostat and RPM	ConA	1-12 h	1 g sim. μg	Decreased IL-2 and IL-2R gene expression
1998	Cooper and Pellis	PBMCs, primary T cells	RWV	PHA	24-72 h	1 g sim. μg	Suppressed proliferation of stimulated cells and reduced IL-2 secretion. Initially suppressed secretion of IFNγ but recovery after 72 h. Reduced CD25 and CD69 surface expression by over 50%. Restoration of proliferation ability in PHA stimulated cultures using submitogenic concentrated PMA
1997	Pellis et al.	PBMCs	RWV	CD3/IL-2	24 h to 9 days	1 g sim. μg	Hampered locomotion into collagen type I matrix

Modified from Hauschild et al. (2014)

CC cell culture control, *ConA* Concanavalin A, *FBS* fetal bovine serum, *HARV* high-aspect ratio vessel, *HEPES* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, *IL* interleukin, *IFN* interferon, *PBL* peripheral blood lymphocytes, *PBMCs* peripheral blood mononuclear cells, *PHA* phytohemagglutinin, *PMA* phorbol-12-myristate-13-acetate, *PKC* protein kinase C, *PARP* poly (ADP-ribose) polymerase, *RCCS* rotary cell culture system, *RWV* rotating wall vessel, *RPM* random positioning machine, *RPMI-1640* Roswell Park Memorial Institute-1640 medium, *TCR* T cell receptor, *TNF* tumor necrosis factor, *n/a* not available

(PHA) stimulated cells compared with cultures in standard cell culture flask with existing cell-substrate interactions (Cooper and Pellis 1998).

Furthermore, in experiments with the ground-based facility RWV, the proliferation of T lymphocytes after stimulation of the receptors CD2/CD28 and CD3/CD28 was completely inhibited (Cooper and Pellis 1998). These stimuli activate the cells without required co-stimulatory signals from cell-cell interaction. Moreover, investigations of human PBMCs and Jurkat T cells in real microgravity showed that cell aggregates and thereby cellular interactions occur despite the absence of gravity (Cogoli-Greuter et al. 1996, 1997; Limouse et al. 1991). Therefore, it has been assumed that changes in the signal transduction are responsible for inhibition of T cell function rather than the absence of cell-cell interactions.

5.1.4 Cytokine Pattern Changes Under Microgravity Condition

In addition to the activation of the T cell receptor complex and the co-receptor CD28, also a third signal via the interleukin-2 receptor (IL-2R) is necessary to fully activate the T cell and therefore to trigger proliferation and differentiation into functional effector T cells. Thus, the reduced functionality of T cells in microgravity might also be due to alterations in cellular IL-2 secretion or IL-2R surface expression, resulting in a disability of the positive regulatory feedback loop.

And in fact, experiments with human PBMCs, which were conducted during several space missions, showed that both the IL-2 secretion and IL-2R expression were greatly reduced in microgravity (Cogoli et al. 1993; Pippia et al. 1996; Hashemi et al. 1999). Additionally, clinostat, RWV, and RPM experiments, where PBMCs and primary human T cells have been exposed to simulated microgravity, confirmed these results (Cooper and Pellis 1998; Hashemi et al. 1999; Risso et al. 2005). Further ground-based experiments demonstrated that already the gene expression of IL-2 and its receptor was inhibited in activated T cells (Walther et al. 1998; Boonyaratanakornkit et al. 2005). However, co-stimulation of the cells with submitogenic concentrations of phorbol-12-myristate-13-acetate (PMA) was able to restore the proliferative response and the expression of IL-2R at the cell's surface (Cooper and Pellis 1998; Hashemi et al. 1999).

Activated T cells also produce interferon-gamma (IFNγ) which is a major proinflammatory and regulatory cytokine. IFNγ plays an essential role in relevant immunological processes such as inflammatory reactions, cell-mediated immunity, and autoimmunity. Studies concerning the IFNγ secretion demonstrated that stimulation of PBMCs with ConA during the spaceflight resulted in an increased IFNγ secretion compared to 1 g ground controls (Chapes et al. 1992), whereas a PMA/ionomycin stimulation of samples isolated from astronauts immediately after their landing led to a significant reduction of IFNγ secretion of $CD4^+$ T lymphocytes. In $CD8^+$ T lymphocytes, however, these remained unchanged (Crucian et al. 2000). In addition, comparison of whole blood analyses from astronauts of short-term (Space Shuttle) and long-term missions (International Space Station (ISS)) showed that a short-term stay in space led to a reduction in the percentage of IFNγ producing T

cells, while after long-term missions the proportion of IFNγ producing T cells remained unchanged (Crucian et al. 2008). Lymphocytes in simulated microgravity provided by RWV exhibited an initial reduction of IFNγ secretion. After 3 days, however, normal levels were restored (Cooper and Pellis 1998).

5.1.5 *Microgravity Affects Cytoskeletal Structures and Cell Motility*

The cytoskeleton is an internal filamentous network of different types of cytosolic fibers: actin filaments, microtubules, and intermediate filaments. This cytoskeletal network is responsible for giving a cell its shape and for generating the required forces for cell motility. But also other biological functions such as cell proliferation, survival, and death are influenced by the cytoskeleton. Since the cytoskeleton participates also in transduction of signals from the receptor at the plasma membrane to the nucleus, these cytoskeletal structures play additionally essential roles in maintaining receptor signaling integrity. Thus, further studies set their focus on the cytoskeletal network.

Indeed, several independent experiments performed under real microgravity conditions demonstrated significant changes in the cytoskeletal network. Analyses of T lymphocytes flown aboard of sounding rockets reported altered tubulin and vimentin structures which appeared in thick bundles (Cogoli-Greuter et al. 1997; Sciola et al. 1999). Jurkat T cells flown on board of the space shuttle Atlantis also showed a modified microtubule network (Lewis et al. 1998). The microtubules were coalesced, did not extend to the cell membrane, and the microtubule organizing center was disorganized.

Cell communication and signal transduction processes are not only affected by cell-to-cell interactions, they are also highly influenced by cell motility. Therefore, the cell motility of lymphocytes was observed under microgravity conditions. These studies showed that the cells were motile; however, their motility did not decrease with increasing duration of stimulation (Cogoli-Greuter et al. 1996, 1997). Furthermore, experiments using the ground-based facility RWV exhibited that after 24 h exposure to simulated microgravity, cell motility of PBMCs was inhibited (Pellis et al. 1997; Sundaresan et al. 2002). However, addition of PMA to the cells could restore cell motility (Sundaresan et al. 2002).

5.1.6 *Distribution of Protein Kinase C (PKC) Isoforms to Destined Cellular Fractions Is Dysregulated*

Since the cytoskeleton also has an impact on proper functioning of signal transduction substantially, restructuring of the cytoskeleton induced by microgravity could also result in hampered intracellular localization of signaling molecules. Accordingly, different PKC isoforms are associated with several cytoskeletal fibers.

Upon T cell activation, under normal circumstances, these PKC isoforms are allocated to destined cellular compartments. In two spaceflight experiments with Jurkat T cells and primary human T cells, it could be shown that the relative distribution of certain PKC isoforms to different cell fractions in the in-flight microgravity samples greatly differed from the 1 g ground controls (Hatton et al. 2002; Schmitt et al. 1996). Further, primary T cells exposed to simulated microgravity in an RWV confirmed these results (Galleri et al. 2002). In addition, another RWV experiment revealed that also mRNA expression and protein expression of specific calcium-independent PKC isoforms in PBMCs were inhibited (Sundaresan et al. 2004).

5.1.7 Lack of Gravity Increases the Rate of Controlled Cell Death (Apoptosis)

Another reason for the reduced proliferative response of T lymphocytes in microgravity might be the initiation of the intracellular death program called programmed cell death or apoptosis. In fact, it could be shown by means of biochemical and microscopic investigations that Jurkat T cells exposed to microgravity demonstrated an increased rate of apoptosis (Cubano and Lewis 2000; Lewis et al. 1998; Battista et al. 2012). This was reflected in the release of apoptosis-related factors such as Fas/APO1 in the cell culture medium after about 2 days aboard different space shuttle flights in cell culture medium (Cubano and Lewis 2000; Lewis et al. 1998). Moreover, exposure of lymphocytes to microgravity resulted in increased DNA fragmentation, poly (ADP-ribose) polymerase (PARP) protein expression, as well as multiplied p53 and calpain mRNA. These changes were associated with an early increase of 5-lipoxygenase (5-LOX) activity (Battista et al. 2012). During an experiment that we conducted during the 8th DLR (German Aerospace Center) parabolic flight campaign, we observed an increase in p53 phosphorylation after 20s of real microgravity (Paulsen et al. 2010). Experiments in simulated microgravity, however, did not confirm microgravity-induced stimulation of apoptosis but revealed that radiation and activation-induced programmed cell death in T lymphocytes was inhibited (Risin and Pellis 2001).

5.1.8 Microgravity Does Not Intervene at the Level of Membrane-Proximal Processes of T Cell Receptor Signaling Within the First Minutes

Up to date it has not yet been revealed whether and how gravitational changes affect the T cell signal transduction, in particular the membrane-proximal and cytoplasmic signal transduction cascades as well as the IL-2/IL-2R activation loop. Although some studies suggest that microgravity intervenes at the level of PKC (Hatton et al. 2002; Schmitt et al. 1996), the addition of PMA to cells exposed to simulated

microgravity restored T cell activation (Cooper and Pellis 1998; Simons et al. 2009), surface receptor expression (Hashemi et al. 1999), and cell motility (Sundaresan et al. 2002). In addition, the first activation signals binding, patching, and capping of ConA on the T cell receptors proceed normally (Cogoli-Greuter et al. 1997). Therefore, it is believed that the gravity-sensitive cellular targets are located more likely upstream of the PKC and downstream of the T cell receptor/CD3 complex.

Thus, in recent studies, we investigated the effects of altered gravity on several key elements involved in the early T cell signaling (Tauber et al. 2013, 2015). For this purpose, primary human $CD4^+$ T lymphocytes were examined under real microgravity conditions aboard the sounding rocket MASER-12 (Tauber et al. 2013) and during the 19th DLR parabolic flight campaign (Tauber et al. 2015). Subsequently, we carried out experiments with the same experimental set-up in simulated microgravity using a fast-rotating 2D-clinostat (Tauber et al. 2015). We analyzed the impact of gravitational changes on the key molecules of the early T cell signaling events in both resting and ConA/CD28-activated $CD4^+$ T lymphocytes. We quantified following signaling components: T cell receptor, membrane-proximal signal proteins LAT and ZAP-70, MAPK, IL-2R, and histone acetylation. Table 5.3 gives an overview of the obtained results.

In addition to the microgravity effects, we investigated the influence of hypergravity during the rocket launch and during the climb of the airplane, as well as the influence of the cultivation of the cells in the experimental hardware. The analyses of the protein level after 6 min of real microgravity during the sounding rocket flight showed no obvious effects on the early signal transduction pathway in $CD4^+$ T lymphocytes. Surprisingly, strong effects of the rocket launch could be observed, which often resulted in a significant reduction of the signal molecules. During the parabolic flight experiment, the 20 s hypergravity phase led to a rapid decrease of CD3 and IL-2R surface expression and reduced p-LAT in nonactivated primary T lymphocytes. The subsequent clinostat experiments showed a decreased CD3 surface expression, reduced ZAP-70 abundance, as well as an increased histone H3-acetylation in activated T lymphocytes after 5 min of clinorotation and a transient downregulation of CD3 and, further, a stable downregulation of IL-2R during 60 min of clinorotation.

However, based on these results it can be assumed that gravitational changes do not intervene at the level of the membrane-proximal key proteins within the first 6 min. The initial primary dysregulation of functional T cell activation will probably occur at the level of regulation of gene expression.

5.1.9 Gravitational Changes Induce Alterations in the Gene Expression Profile

The phenomenological characteristics of reduced T cell activation caused by microgravity are now well described. The exact underlying molecular mechanisms, however, are still unknown. So, during the last decade, several studies focused on the

Table 5.3 Overview of qualitative changes of selected proteins involved in T cell activation of primary human $CD4^+$ T lymphocytes induced by altered gravity during the sounding rocket flight of MASER-12, during the 19th DLR parabolic flight campaign, and by 2D-clinorotation

	Signaling molecules					
	CD3	ZAP-70	LAT (pY171)	P-p44/42 MAPK	Acetyl-histone H3	IL-2R
19th DLR parabolic flight campaign						
Nonactivated T lymphocytes						
1 g in-flight vs. 1 g hardware controls	–	–	–	–	–	–
1.8 g hypergravity vs. 1 g in-flight	↓**	–	↓*	–	–	↓**
Microgravity vs. 1 g in-flight	↓*	–	↓*	–	–	↓*
Microgravity vs. 1.8 g hypergravity	–	–	–	–	–	–
MASER-12 sounding rocket						
Controls						
1 g hardware controls vs. cell culture controls	↓**	↓**	↓**	–	↑**	–
Hypergravity (rocket launch) vs. 1 g hardware controls	↓**	↓*	–	↓**	↓**	↓**
Nonactivated T lymphocytes						
Microgravity vs. 1 g in-flight	–	–	–	↓*	–	–
ConA/CD28 activated T lymphocytes						
Microgravity vs. 1 g in-flight	–	–	–	–	–	–
Fast-rotating 2D-clinostat						
Nonactivated T lymphocytes						
Hardware controls vs. cell culture controls	–	–	–	–	–	–
5 min clinorotation vs. 1 g controls	–	–	–	–	↑*	–
ConA/CD28 activated T lymphocytes						
5 min clinorotation vs. 1 g controls	↓**	↓*	–	–	↑*	–
15 min clinorotation vs. 1 g controls	↓*	nd	nd	Nd	nd	–
30 min clinorotation vs. 1 g controls	–	nd	nd	Nd	nd	↓*
60 min clinorotation vs. 1 g controls	–	nd	nd	Nd	nd	↓*

Modified from Tauber et al. (2015)

Hardware control: cells were cultured without changing gravity conditions in the corresponding hardware; *Cell culture control*: optimal culture conditions prior to fixation; *1 g in-flight*: cells were fixed before onset of the parabola during the parabolic flight or cells were reexposed to 1 g at a reference centrifuge aboard the sounding rocket MASER-12; *Hypergravity*: cells were fixed after 1.8 g phase during the climb of the aircraft or after the rocket launch of MASER-12; *Microgravity*: during parabolic flight or suborbital sounding rocket flight; *Clinorotation*: cells were exposed simulated microgravity by means of 2D-clinostats. Cells were immunocytochemically stained, staining was quantified using flow cytometry analysis, and the relative fluorescence intensity was calculated. ↓ indicates downregulation, ↑ indicates upregulation, – indicates no significant difference within the compared groups

nd not determined

*$p<0.05$; **$p<0.01$

investigation of the impact of altered gravity on gene transcription (Thiel et al. 2012; Chang et al. 2012; Boonyaratanakornkit et al. 2005; Ward et al. 2006; Sundaresan and Pellis 2009; Kumari et al. 2009; Lewis et al. 2001).

The evaluations of genome-wide gene expression analyses of T cells revealed that both in real and simulated microgravity, the expression of very early genes, which are primarily regulated by the transcription factors NF-kB, CREB, ELK, AP-1 and STAT, were downregulated in comparison to 1 g controls (Chang et al. 2012; Boonyaratanakornkit et al. 2005). The observed changes in gene expression induced by altered gravity include a number of genes which are associated with responses to cell stress (Sundaresan and Pellis 2009), cell proliferation and differentiation (Ward et al. 2006; Sundaresan and Pellis 2009; Boonyaratanakornkit et al. 2005), cell cycle regulation (Kumari et al. 2009; Thiel et al. 2012), protein folding (Ward et al. 2006), DNA repair (Kumari et al. 2009), transport and degradation (Ward et al. 2006), apoptosis (Ward et al. 2006; Kumari et al. 2009; Lewis et al. 2001), and the cytoskeleton (Lewis et al. 2001). These results show that modulation of gene expression in reduced gravity covers a wide spectrum.

In an RWV study, alterations in the microRNA (miRNA) profiles of human lymphocytes exposed to simulated microgravity for 1–3 days were observed (Girardi et al. 2014). The examinations identified 42 differentially expressed miRNA whereof the upregulated miR-9-5p, miR-9-3p, and miR-155-5p, and the downregulated miR-150-3p and miR-378a-3p were the most dysregulated ones. Further, miRNA-correlated genes whose expression level was also significantly altered by simulated microgravity were investigated. Thus, several miRNA-mRNA pairs, which are involved in biological processes such as immunity and inflammatory response, cell proliferation, and apoptosis, were determined.

In a most recent study, Millie Hughes-Fulford and her team discovered the suppressed expression of the miRNA miR-21 due to altered gravity after 1.5 h T cell activation during spaceflight (Hughes-Fulford et al. 2015). Furthermore, microarray analysis showed that 85 genes associated with T cell signaling were significantly downregulated under microgravity conditions compared to 1 g in-flight controls. Of these gravity-sensitive genes, 17 were defined as targets of miR-21 whereof 5 genes are biologically confirmed targets and are under normal circumstances upregulated in parallel with miR-21. Therefore, it can be assumed that altered gravity influences T cell activation not only by transcription promotion but also by repressing translation via noncoding RNA mechanisms.

Further experimental studies with primary human T cells disclosed microgravity-induced epigenetic changes in DNA methylation and chromatin histone modifications (Singh et al. 2010). Such epigenetic mechanisms regulate and modify the activation of certain genes and therefore lead to differential expression of mRNA. Experiments that we conducted during several parabolic flight experiments (9th, 10th, and 13th DLR and 45th ESA Parabolic Flight Campaign) have revealed an association between microgravity-induced differentially mRNA expression and altered histone acetylation (Thiel et al. 2012).

5.2 Humoral Immunity

Apart from the cellular components, the adaptive immune system also includes the humoral immunity [see also Chap. 1]. However, the humoral immunity has not been investigated to that extent of which the cell-mediated immunity has been investigated. Short-term spaceflight has resulted in no change in levels of plasma immunoglobulins (Voss 1984; Stowe et al. 1999; Rykova et al. 2008), whereas, long-term spaceflight led to different results. Studies of cosmonauts during spaceflight have shown that immunoglobulin G (IgG) levels were unchanged, whereas IgA and IgM levels were in some cases increased (Konstantinova et al. 1993). In another study, immunological investigations comparing the preflight with the postflight situation indicated that the total amounts of serum IgA, IgG, and IgM were unchanged after long-term missions (Rykova et al. 2008). Therefore, the humoral immune responses may not be as sensitive to altered gravity as are cell-mediated immune responses.

5.3 Conclusion

These numerous studies carried out with T lymphocytes in microgravity have clearly shown that already individual cells are sensitive to changes in gravity. In addition, these experiments conducted under real and simulated microgravity conditions contributed greatly to our current knowledge of how changes of the gravitational force affect basic cellular mechanisms. The influence of microgravity on the function of T lymphocytes is reflected in a variety of cellular responses, which can be grouped into different categories displayed in Table 5.4 and Fig. 5.1.

Since the simulation of the microgravity yielded comparable results to real microgravity experiments (Herranz et al. 2013), it was possible to perform a large number of simulation experiments which would not have been feasible in this scale only by space experiments. So far, however, it has not been possible to formulate a generally accepted hypothesis from these various effects and to further locate any possible primary mechanism that underlies the effects of altered gravity on immune cells.

To date, in vitro research has mainly focused on the impact of altered gravity of T helper cells. In a recent in vivo study (Chang et al. 2015), in which the influence of microgravity was examined for tolerance induction, transgenic mice were exposed to microgravity for 15 days during spaceflight. In this experiment, it could be shown for the first time that the immune tolerance is inhibited in space. Moreover, it provides indications of a potential key role of regulatory T cells.

On closer inspections of the studies reviewed in this chapter, the experimental conditions vary widely from study to study. For example, stimuli used for the T cell activation ranged from mitogens over calcium ionophores up to antibodies against

Table 5.4 Overview of the observed effects of altered gravity on human T cells cultured in vitro

Category	Effects
Apoptosis	Time-dependent increase in apoptosis-related factors (Cubano and Lewis 2000; Lewis et al. 1998). Increase of DNA fragmentation, PARP protein expression, and p53 and calpain mRNA levels; early increase of 5-LOX activity (Battista et al. 2012). Increased p53 phosphorylation (Paulsen et al. 2010). Inhibition of induced programmed cell death (Risin and Pellis 2001). Induction of DNA damage (Kumari et al. 2009)
1.1 Cell cycle regulation	Enhanced p21 protein expression, less cdc25C protein expression, and enhanced phosphorylation of cyclinB1 (Thiel et al. 2012)
1.2 Cell motility	Inhibition of PBMC locomotion (Pellis et al. 1997; Sundaresan et al. 2002)
1.3 Chromosomal instability	DNA replication was inhibited, therefore structural chromosome instability was enhanced (Wei et al. 2014)
1.4 Cytokine secretion	Suppressed IL-2 secretion (Cooper and Pellis 1998; Limouse et al. 1991; Risso et al. 2005; Crucian et al. 2000) and increased IFNγ secretion (Chapes et al. 1992). Reduced IFNγ production by $CD4^+$ T cell subset (Crucian et al. 2000). Reduced percentage of T cell subsets producing IL-2 and/or IFNγ during short resp. long-term spaceflight (Crucian et al. 2008). Strongly increased IFNγ (Bechler et al. 1992; Cogoli et al. 1993) and IL-2 (Cogoli et al. 1993) production of cells attached to microcarrier beads. Suppressed IFNγ secretion, but reversible (Cooper and Pellis 1998)
1.5 Cytoskeleton	Structural changes of intermediate filaments of vimentin and of the microtubule network (Lewis et al. 1998; Cogoli-Greuter et al. 1997; Sciola et al. 1999; Schatten et al. 2001)
1.6 Epigenetic changes	Differential DNA methylation and chromatin histone modification (Singh et al. 2010)
1.7 Gene expression	Differential expression of genes involved in DNA repair, cell cycle, cell growth, metabolism, signal transduction, adhesion, transcription, apoptosis, tumor suppression, immune response, cell activation, proliferation and differentiation, protein folding, transport and degradation, cytoskeleton, stress response, and apoptosis (Thiel et al. 2012; Chang et al. 2012; Boonyaratanakornkit et al. 2005; Ward et al. 2006; Sundaresan and Pellis 2009; Kumari et al. 2009; Lewis et al. 2001)
1.8 miRNA expression	Altered miRNA expression influencing genes involved in regulation of NF-kB-related signaling network (Mangala et al. 2011). 42 differentially expressed miRNAs in RWV-incubated lymphocytes whereof miR-9-5p, miR-9-3p, miR-155-5p, miR-150-3p, and miR-378-3p were the most dysregulated (Girardi et al. 2014). Suppressed expression of miR-21 after 1.5 h T cell activation correlating with 17 downregulated genes defined as targets of miR-21 (Hughes-Fulford et al. 2015)
1.9 Mitochondria distribution	Mitochondria clustering and morphological alterations of mitochondrial cristae (Schatten et al. 2001)
1.10 PKC distribution	Altered distribution of PKC isoforms to particular cell fractions (Hatton et al. 2002; Schmitt et al. 1996; Galleri et al. 2002)

(continued)

Table 5.4 (continued)

Category	Effects
1.11 Signaling	Hypergravity-induced dysregulation of several key signal proteins involved in early TCR signaling (Tauber et al. 2013, 2015). Enhanced phosphorylation of the MAP kinases and inhibition of NF-kB translocation into nucleus during simulated microgravity (Paulsen et al. 2010). Higher calcium concentration in CD69/PMA stimulated cells (Risso et al. 2005). Calcium signaling remains active, activation of fos and NF-kB is inhibited (Morrow 2006)
1.12 Surface receptor expression	Reduced surface expression of CD25 and CD69 (Cooper and Pellis 1998; Hashemi et al. 1999). Retarded TCR internalization (Hashemi et al. 1999). Suppressed CD3 and IL-2R-surface receptor expression (Tauber et al. 2015)

Modified from Hauschild et al. (2014)

surface receptors. Moreover, different basal media for culturing of the T cells were used which were supplemented in most cases also with different additives in varying concentrations. In nearly all present studies, fetal bovine serum (FBS) has been added to the T cell culture medium ranging from 10 to 20 %.

However, the chemical composition of serum is highly variable and ill defined. Since it contains a large number of constituents, including biomolecules with a variety of growth-promoting and growth-inhibiting activities, the use of serum in cell culture media has obviously considerable effects on phenotypic and genotypic cell stability. Furthermore, the concentrations of the components vary not only from manufacturer to manufacturer but also from batch to batch. Thus, cell signaling, cell proliferation, and differentiation and of course gene expression are influenced by the varying components in different serum used for different experiments.

Therefore, the comparability between the various studies that have been carried out, in order to obtain an overall picture and to locate possible fundamental primary microgravity-induced mechanisms, is not reliable. Nowadays, this lack of standardization has to be regarded as unacceptable. Maintaining high-level standards is of fundamental importance for ensuring good scientific practice in order to maximize reproducibility, reliability, acceptance, and successful implementation of results. Moreover, since scientific research in the area of gravitational science is extremely expensive and elaborate, their resources should be spent wisely. Therefore, in order to achieve the highest level of reliability and comparability of the results, gravitational-related immunobiological research should benefit to a large extent from the latest technology for the standardization of cell and tissue cultures and the development of chemically defined media.

The knowledge of the effects of gravitational changes on T cell regulation and the identification of gravity-sensitive cell responses will help to understand the molecular mechanisms of the inhibited immune cell function in altered gravity and thus new targets for therapeutic or preventive interventions with respect to the immune system of astronauts during long-term space missions may be developed (Ullrich and Thiel 2012).

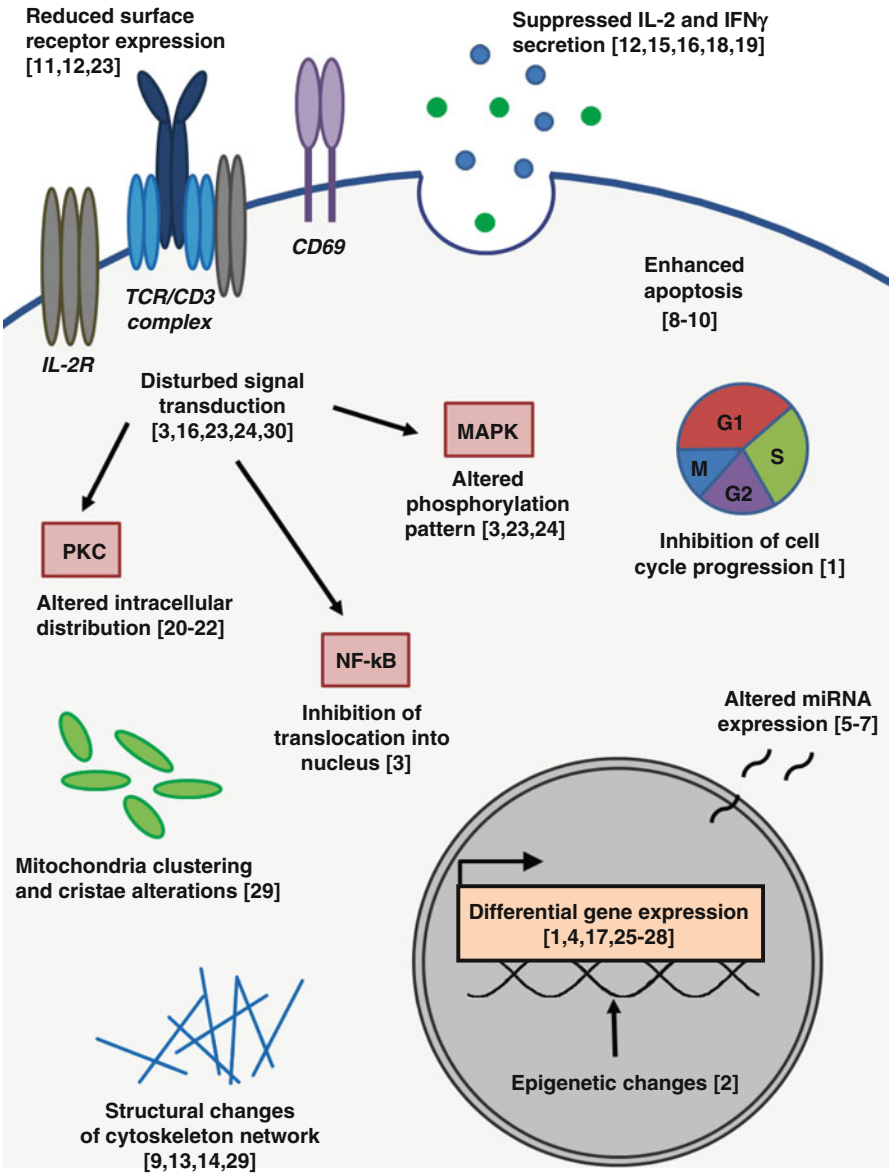

Fig. 5.1 Schematic summary of the influence of microgravity on T lymphocyte function (Modified from Hauschild et al. 2014). *1* Thiel et al. (2012), *2* Singh et al. (2010), *3* Paulsen et al. (2010), *4* Chang et al. (2012), *5* Mangala et al. (2011), *6* Girardi et al. (2014), *7* Hughes-Fulford et al. (2015), *8* Cubano and Lewis (2000), *9* Lewis et al. (1998), *10* Battista et al. (2012), *11* Hashemi et al. (1999), *12* Cooper and Pellis (1998), *13* Cogoli-Greuter et al. (1997), *14* Sciola et al. (1999), *15* Limouse et al. (1991), *16* Risso et al. (2005), *17* Boonyaratanakornkit et al. (2005), *18* Crucian et al. (2000), *19* Crucian et al. (2008), *20* Hatton et al. (2002), *21* Schmitt et al. (1996), *22* Galleri et al. (2002), *23* Tauber et al. (2015), *24* Tauber et al. (2013), *25* Ward et al. (2006), *26* Sundaresan and Pellis (2009), *27* Kumari et al. (2009), *28* Lewis et al. (2001), *29* Cogoli et al. (1985a, b), *30* Simons et al. (2010)

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