Chapter 4
Cellular Effects of Altered Gravity on the Innate Immune System and the Endothelial Barrier

Svantje Tauber and Oliver Ullrich

The innate immune system is of essential importance to protect the human body from infection as it recognizes, inactivates, and kills intruding pathogens. It comprises different types of leukocytes, each having specialized functions to dispose pathogens. Their capacities cover phagocytosis, secretion of cytokines to recruit other cells, oxidative burst, and secretion of toxins. During elongated spaceflight, a pronounced immune dysfunction has been observed in astronauts that becomes manifest in an enhanced susceptibility to infections by bacteria, viruses, and fungi (Sonnenfeld 2002). This immunodeficiency has inspired curiosity about possible effects of altered gravity conditions on immune cells, and numerous studies have been performed since the 1970s to address the effects of altered gravity on immune cells as a possible underlying mechanism of space-induced immunodeficiency. This chapter will focus on the effects of altered gravity on the cells of the innate immune system, while the effects on the adaptive immune system are discussed in Chap. 3 [part 4].

S. Tauber (✉)
Institute of Anatomy, University of Zurich,
Winterthurerstrasse. 190, CH-8057 Zurich, Switzerland

Institute of Mechanical Engineering, Department of Machine Design,
Otto-von-Guericke University Magdeburg, Universitätsplatz 2,
39106 Magdeburg, Germany
e-mail: svantje.tauber@uzh.ch

O. Ullrich
Institute of Anatomy, University of Zurich,
Winterthurerstrasse. 190, CH-8057 Zurich, Switzerland

Institute of Mechanical Engineering, Department of Machine Design,
Otto-von-Guericke University Magdeburg, Universitätsplatz 2,
39106 Magdeburg, Germany

Space Life Sciences Laboratory (SLSL), Kennedy Space Center, 505 Odyssey Way,
Exploration Park, FL 32953, USA

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During acute inflammation, leukocytes, especially granulocytes, need to interact highly coordinated with the endothelial cells (ECs) of the vascular system to reach the sites of infection. The vascular endothelium is composed of a layer of closely connected ECs and separates the blood from the surrounding tissue. This endothelium plays a fundamental role in tissue homeostasis as it regulates vasoconstriction/vasodilatation and builds a semipermeable barrier that regulates blood-tissue exchange of plasma, molecules, and cells. ECs have mechanosensory properties; they can react to fluid shear stress (Topper and Gimbrone 1999) and pressure (Fu and Tarbell 2013). Additionally the endothelium builds a physical barrier against pathogens that have entered the circulation and hinders them to infiltrate the surrounding tissues. For leukocytes, the endothelial barrier provides an inducible and highly specific permeability: during inflammation ECs are activated, meaning that the expression pattern of surface molecules is altered which enables leukocytes to roll along and subsequently bind to the endothelium. These changes allow leukocytes to cross the endothelial barrier, a process called diapedesis, and migrate through tissues to the sites of infection (Yuan and Rigor 2010). Junctional complexes between adjacent cells play a major role in leukocyte extravasation and vascular permeability; their composition is modulated dynamically (Aghajanian et al. 2008). Dysfunction of the endothelial barrier is involved in many pathological circumstances such as the extravasation during tumor metastasis, thrombosis, inflammation, diabetes mellitus, trauma, epilepsy, sepsis, and multiple sclerosis (Yuan and Rigor 2010; Reymond et al. 2013). Additionally to the already mentioned immune dysfunction (Sonnenfeld 2002) and the well-known dystrophic effects on muscle and bone, astronauts suffer from cardiovascular issues due to vascular impairment during spaceflight (Convertino 2009). ECs are of central importance for both cardiovascular homeostasis and inflammatory processes. Taking into account that ECs can sense mechanical stimuli and convert them into cellular signals (Feletou et al. 2010; Busse and Fleming 2003), the question arises if ECs are sensitive to gravitational changes and possibly contribute to the physiological dysfunctions observed during spaceflight.

Numerous studies have been conducted to evaluate and to understand the effects of altered gravity on cells of the innate immune system and ECs (Maier et al. 2015). Therefore, the blood of astronauts and participants of parabolic flights has been investigated, and many in vitro studies with isolated cells in real and simulated microgravity have been performed. Various effects of microgravity and hypergravity were observed comprising very basal cellular functions such as proliferation as well as effector functions such as oxidative burst, adhesion, locomotion, and cytokine secretion. Table 4.1 summarizes the effects of altered gravity on cells of the innate immune system and on ECs.

The results obtained in different studies might seem partly conflicting. To interpret the data, it must be kept in mind that they were obtained partly in real microgravity and partly from platforms that provide simulated microgravity, which can only model some aspects of real microgravity. Another source of discrepancies between experimental outcomes may be the use of cell models from different species and the differences between primary cells and cell lines. For ECs, the origin of the cells with respect to aortic or venular location in the vascular system might also have an influence on the experimental outcome. Therefore, results should be interpreted with respect to their particular experimental setup.

Table 4.1 Effects of altered gravity on cells of the innate immune system and endothelial cells

Cell type/focus of investigation	Cell model	Research platform	Findings	Reference
Monocytes/ intracellular signal transduction	U937	Space shuttle flight	The subcellular distribution of PKC was altered in microgravity samples compared to on-board 1 g samples: with increasing g-force, the level of PKC in the nuclear fraction decreased while it increased in the cytosolic fraction. The synthesis of interleukin-1β was decreased in microgravity samples	Schmitt et al. (1996)
	U937 monocytic cells	Spaceflight	The translocation of PKC from the cytosol to the particulate fractions showed an altered as compared to controls on the ground. Additionally enhanced binding of phorbol ester to PKC was observed in-flight, while hypergravity (1.4 g) led to decreased binding	Hatton et al. (1999)
	U937 monocytic cells	Spaceflight	Translocation of the PKC isoforms PKC β II, delta, and epsilon in response to phorbol ester was decreased compared to 1 g but was increased by hypergravity (1.4 g)	Hatton et al. (2002)
	Peripheral blood monocytes	Sounding rocket, spaceflight	In LPS-stimulated cells microgravity led to an impairment of Jun-N-terminal kinase activation compared to on-board 1 g controls. In contrast, activation of p38 MAP kinase was not altered	Verhaar et al. (2014)
	U937 monocytes	Parabolic flight	Microgravity led to enhanced overall tyrosine phosphorylation and activation of c-jun in non-stimulated U937 cells and to decreased overall tyrosine phosphorylation and reduced activation of c-jun in PMA-stimulated cells	Paulsen et al. (2010)
Monocytes/ differentiation	Bone marrow cells, murine	Spaceflight	In a subpopulation of murine bone marrow cells that contained macrophage-like cells, 13 days of spaceflight led to decreased expression of Ly6C, c-Fos, CD44 (high), and Ly6G and an increased expression of F4/80	Ortega et al. (2009)
	Monocytes/ macrophages	International space station	The expression of genes involved in the differentiation process of monocytes into macrophages is altered in microgravity	Hughes-Fulford et al. (2008)

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Table 4.1 (continued)

Cell type/focus of investigation	Cell model	Research platform	Findings	Reference
Monocytes, proliferation, and cell cycle control	U937 monocytic cells	RWV	Cell proliferation was reversibly decreased in microgravity	Cotrupi and Maier (2004)
	U937	RWV	Microgravity leads to slower growth, a decreased level of cdc25B, alteration in cytokine secretion, and decreased proteasome activity	Maier (2006)
	THP-1 monocytic cells	RCCS	Microgravity for 24 h leads to a decrease in proliferation and to an inhibition of LPS-induced expression of tissue factor mRNA	Yu et al. (2011)
Monocytes, oxidative burst	RAW 264·7, macrophage cell line, murine	RWV bioreactor	4 days of culture in microgravity and stimulation with LPS/IFN-γ on day 2 led to a decrease in the production of nitric oxide of 65 % and to a decrease of cytokine production (TNF-α, IL-6, IL-12) of 80 % compared to 2D cultured cells	Hsieh et al. (2005)
	NR8383 rat alveolar macrophages	Clinostat (2D), parabolic flight, centrifuge	The release of ROS was decreased by real and simulated microgravity and was increased in hypergravity in a rapidly responding and reversible manner	Adrian et al. (2013)
	NR8383 rat alveolar macrophages	Fast-rotating clinostat	Macrophages that were stimulated with zymosan, curdlan, or lipopolysaccharide produced significantly less ROS when exposed to microgravity compared to normal gravity. Reduced phosphorylation of spleen tyrosine kinase (Syk) was observed. The translocation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) to the nucleus was not altered	Brungs et al. (2015)
Monocytes/ phagocytosis	Monocytes from blood of astronauts	Four space shuttle missions	Spaceflight (5–11 days) decreased the percentage of phagocytizing monocytes and the phagocytic index of monocytes as measured by the ability to engulf bacteria. The expression of the surface markers CD32 and CD64 was altered	Kaur et al. (2005)

Monocytes/ interleukin secretion	Jurkat T cells and THP-1 monocytes	Biosatellite Cosmos 2044	T lymphocytes and monocytes in co-culture respond to T cell activation with normal secretion of IL-2 and IL-1, respectively, under microgravity. In contrast, if cultured separately and stimulated, IL-2 and IL-1 secretion were reduced in microgravity	Limouse et al. (1991)
	Peripheral blood monocytes	Spacelab	Peripheral blood monocytes responded with a strong decrease in interleukin-1 secretion to weightlessness in an experiment with T lymphocytes and monocytes which were stimulated with ConA	Cogoli et al. (1993)
	Human peripheral blood mononuclear cells	Spaceflight (Biorack facility), clinorotation	During clinorotation and real microgravity, PBMCs reacted with a decrease in IL-2 receptor expression to stimulation with anti-CD-3. Upon stimulation with anti-CD-3 (leading to cell-cell contact between T cells and monocytes), the level of synthesized IL-1 by monocytes stayed unaffected during 24 h clinorotation	Hashemi et al. (1999)
	Monocytes from astronaut blood	Spaceflight	In peripheral monocytes from astronauts, reduced levels of CD62L and HLA-DR were measured after 13–16 days of spaceflight. Cells responded to ex vivo LPS stimulation with decreased expression of IL-6, TNF-α, and IL-10 and increased expression of IL-1b. The expression of IL-8 was found to be regulated in either direction, dependent on the space flight mission	Crucian et al. (2011)
	B6MP102, cultured murine bone marrow macrophage cell line	Spaceflight	In a murine bone marrow macrophage cell line LPS-induced secretion of tumor necrosis factor-α and interleukin-1 was higher compared to controls on earth	Chapes et al. (1994)
	RAW264.7 cells and primary mouse macrophages	RCCS	In mouse macrophages cultured for 24 in microgravity, the expression of LPS-induced TNF-α was markedly decreased compared to 1 g culture. Phosphorylation of IKK and JNK and nuclear translocation of NF-kB as well as TNF-α mRNA stability were not altered, but heat shock factor-1 (HSF1), a repressor of TNF-α promoter, was upregulated	Wang et al. (2014)

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Table 4.1 (continued)

Cell type/focus of investigation	Cell model	Research platform	Findings	Reference
Monocytes/ cytoskeleton and locomotion	J-111, adherent monocyte cell line	RPM	The ability for locomotion was inhibited, and actin, tubulin and vinculin were altered	Meloni et al. (2006)
	Monocytes J-111	International space station	The ability of monocytes to migrate was decreased in microgravity samples compared to 1 g in-flight and ground controls. The cytoskeletal architecture was markedly disrupted, as the distribution of F-actin, β-tubulin, and vinculin structures was changed. For F-actin fibers, quantitative analysis showed a significant reduction	Meloni et al. (2011)
Macrophages	Primary mouse macrophages	RCCS	Microgravity for 24 h led to increased levels of arginase mRNA and protein levels, enhanced expression of C/EBPβ (a transcription factor which is relevant for arginase transcription), and activation of p38. LPS-stimulated primary mouse macrophages reacted with increased levels of IL-6 and decreased levels of IL-12B	Wang et al. (2015)
NK-Cells	NK cells from human blood, in vitro	International space station and clinostat	24 h of real microgravity and clinorotation did not lead to alterations of cytotoxic activity of NK cells toward target cells. The interferon production of NK cells upon binding of target cells in real microgravity did not alter from that of ground controls	Buravkova et al. (2004)
	NK cells from human blood, ex vivo expanded	RWV	Microgravity for 48 h led to a decrease in cytotoxicity which could be counteracted by IL-15 alone or in combination with IL-12. Increased levels of apoptosis and necrosis and decreased expression levels of IFN-γ and perforin were observed. The NK cell surface receptors NKG2A and NKG2D were expressed at reduced levels after exposure to microgravity; expression of NKp30 and NKp44 was not altered	Li et al. (2013)
	Peripheral blood monocytic cells, human	RWV	Natural killer activity and lymphokine-activated killing of PBMCs upon stimulation with IL-2 were not different in cells exposed to microgravity and 1 g controls. Nevertheless, the stimulation-induced upregulation of IL-2 receptor α chain (CD25) was decreased under microgravity, and the secretion of the secondary cytokines of IFN-γ, IL-1β, and TNF-α was reduced	Licato and Grimm (1999)

	NK cells from astronaut blood	Spaceflight	After a 9-day space mission, the number of NK cells in the blood of astronauts was decreased, while it was unchanged after a 16-day mission	Stowe et al. (2003)
	NK cells from astronaut blood	Spaceflight	After 7 days of spaceflight, NK cells of astronauts displayed a decreased killer activity and decreased induced interferon production on day one after return from space	Talas et al. (1983)
	NK cells from astronaut blood	Spaceflight	In a cosmonaut the ability to bind target cells and the percentage of NK cells were decreased after spaceflight of 21 days	Konstantinova et al. (1995)
	NK cells from astronaut blood	Spaceflight	During four space shuttle missions that lasted 10–18 days, no differences in NK cell percentage was observed in the blood of 27 astronauts	Crucian et al. (2000)
Neutrophil granulocytes, PMNs	*Propionibacterium acnes*-induced peritoneal inflammatory cells	Parabolic flight	Superoxide-anion ($O2^{-}$) production by peritoneal inflammatory cells was increased fourfold by microgravity	Fleming et al. (1991)
	Neutrophil granulocytes from blood of astronauts	Space shuttle flight	After 8–15 days of spaceflight, the number of neutrophil granulocytes in the blood of astronauts was increased by 50 %. In an optimal dose response chemotactic assay, the neutrophil granulocytes showed a tenfold decrease after spaceflight indicating an enhanced chemotactic activity	Stowe et al. (1999)
	Polymorphonuclear leukocytes (PMNs) from the blood of astronauts	Space shuttle flight	The number of PMNs in the blood of astronauts was increased after a 16-day space shuttle mission	Stowe et al. (2003)
	Neutrophil granulocytes from blood of astronauts	Space shuttle flight	After spaceflight of 5–11 days, the number of neutrophil granulocytes in the blood of astronauts increased by 85 %	Kaur et al. (2004)

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Cell type/focus of investigation	Cell model	Research platform	Findings	Reference
	PMNs from the blood of parabolic flight participants	Parabolic flight	In the blood of volunteers, the number of PMNs was enhanced significantly post flight. Whereas the spontaneous production of hydrogen peroxide was not altered, the capability to produce it upon stimulation with fMLP, fMLP, and TNF-α, calcium ionophore or PMA was markedly increased. No differences in the ability of the PMNs to adhere and to phagocyte were detected. IL-8 and granulocyte colony-stimulating factor (G-CSF) were found to be elevated in the cell plasma	Kaufmann et al. (2009)
	PMNs from the blood of parabolic flight participants	Parabolic flight	In PMNs from participants of parabolic flight, the potency of adenosine to control the release of hydrogen peroxide was significantly increased 48 h after the flight compared to the status before flight. This effect was due to an upregulation of the adenosine A2(A) receptor function	Kaufmann et al. (2011)
Dendritic cells	CD34+progenitor cells from peripheral human blood, generated in vitro into dendritic cells	RCCS	Dendritic cells were generated in static culture or in a RCCS. The latter were less in number, had decreased capability for phagocytosis and a decreased density of HLA-DR on their surface, and were less effective in antigen-induced responses	Savary et al. (2001)
Endothelial cells	Human umbilical vein endothelial cells (HUVEC), primary	RWV bioreactor	Microgravity led to a reversible stimulation of cell growth, an enhanced expression of heat shock protein 70, and a decreased level of IL-1 α. Furthermore remodeling of the cytoskeleton and, after several days, a decrease of actin were observed	Carlsson et al. (2003)
	Human umbilical vein endothelial cells (HUVEC), primary	RWV bioreactor, RPM, Centrifuge (MidiCAR3.5 xg)	Microgravity led to enhanced growth, enhanced NO production, while migration was not affected. Remodeling of the actin cytoskeleton was observed with a decrease of the amount of actin protein. Hypergravity (MidiCAR3.5 xg) for 24–48 h led to enhanced migration and enhanced NO synthesis in HUVEC cells. After 96 h the distribution of actin fibers was altered toward a perinuclear gathering, but the amount of actin protein was not changed	Versari et al. (2007)

	Human umbilical vein endothelial cells (HUVEC), primary	Spaceflight	After 10 days of spaceflight 1023 genes were modulated significantly as compared to 1 g ground controls. Modulated genes are involved in oxidative phosphorylation, cell adhesion, cell cycle, stress response, and apoptosis. The secretion of IL-1α and IL-1β was enhanced, and nitric oxide production was not altered	Versari et al. (2013)
	Human umbilical vein endothelial cells (HUVEC), primary	RWV bioreactor	Cells grew faster than control cells for up to 8 days. They produced more prostacyclin and NO than controls (vasodilators). Production of metalloproteinases was not changed; productions of TIMPs was enhanced	Carlsson et al. (2002)
	Human umbilical vein endothelial cells (HUVEC), primary	RPM, In vivo hind limb suspension	24 or 48 h culture in a RPM led to significantly decreased expression of IL-6 and TNF-α gene expression. The gene expression and the surface presence of ICAM-1, VCAM-1, and E-selectin were significantly decreased. The effects were reversible by addition of mechanical loading during the mechanical unloading period. In vivo hindlimb suspension led to an increased expression in eNOS and caveolin-1 and caveolin-2 in mouse aortas. Mechanical unloading also led to cytoskeletal changes: decreased length and width and disorganization of the F-actin network, and perinuclear clustering of the fibers was observed	Grenon et al. (2013)
	Human umbilical vein endothelial cells (HUVEC), primary	Spaceflight	12 days of spaceflight led to cytoskeletal lesions and increased cell membrane permeability. In readapted cells which were cultivated after retrieval, persisting cytoskeletal changes, decreased cell growth, and decreased metabolism were observed	Kapitonova et al. (2012)
	Human umbilical vein endothelial cells (HUVEC), primary	NASA RCCS, parabolic flight	24 h in the RCCS led to significantly increased surface expression of ICAM-1 compared to 1 g controls in TNF-α-activated cells. In real microgravity during parabolic flight, an upregulation of ICAM-1 was observed already after 20 s as compared with in-flight 1 g controls. Simulated microgravity (5 min -24 h) led to changes in the distribution of F-actin and altered clustering of ICAM as shown by immunocytochemistry. ICAM-1 mRNA expression was enhanced compared to the controls after 30 min and 1 h, while it was equal to the control after 24 h. Similar results were obtained for VCAM-1	Zhang et al. (2010)

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Cell type/focus of investigation	Cell model	Research platform	Findings	Reference
	Human umbilical vein endothelial cells (HUVEC), primary	RPM	2-D proteome analysis of cellular secretome revealed that after 96 h simulated microgravity, the secretion of proteins relevant for the regulation of cytoskeleton assembly was altered. IL-1α and IL-8 (pro-inflammatory) secretion was inhibited; RANTES and Eotaxin (leukocyte recruitment) secretion was increased. Secretion of the pro-angiogenic factor bFGF was decreased	Griffoni et al. (2011)
	Human umbilical vein endothelial cells (HUVEC), primary	Spaceflight, ISS	8 days in real microgravity led to enhanced levels of IL-6, sICAM-1, and e-selectin in cell supernatant indicating endothelial activation. The mRNA expression of IL-6, ICAM, and VCAM-1 in the cells was increased	Muid et al. (2010)
	Human umbilical vein endothelial cells (HUVEC), primary	Clinorotation (2D)	In TNF-α stimulated cells 18 h of microgravity led to an increase of ICAM-1 expression but a decrease of e-selectin and VCAM-1. In non-stimulated cells exposure to microgravity also enhanced the expression of ICAM-1 and had no effect on e-selectin and VCAM-1. In a co-culture of HUVECs and lymphocytes, the adhesion of phorbol ester-stimulated lymphocytes to endothelial cells was enhanced by 18 h of clinorotation, while the adhesion of non-stimulated lymphocytes was not altered or even slightly lower	Buravkova et al. (2005)
	Human umbilical vein endothelial cells (HUVEC), primary	Centrifuge, 3 g	In HUVECs hypergravity (3 g) for 48 h led to a shift in the cell cycle distribution toward the G(0)/G(1) phase. Calveolin1 gene expression was enhanced and intracellular distribution of caveolae was increased. COX-2 expression, NO production, and prostacyclin (PGI2) production were upregulated	Spinsi et al. (2003)

	EA.hy926, cell line	RPM	Microgravity led to increase of collagen types I and III. After an initial upregulation with a maximum after 10 min, the expression levels of osteopontin and TGF-β1 had declined to the level of the controls at day 10. After 10 days of microgravity Caspase-3 protein content was higher, and the number of Bax and Bcl-2 proteins positive cells was increased, whereas fewer cells were positive for Fas protein. Microgravity-induced cytoskeletal alterations included changes in α- and β-tubulins and F-actin fibers. Secretion of the soluble factors neurotrophic factor, ET-1, tissue factor, and VEGF was decreased in microgravity after 10 days of microgravity	Infanger et al. (2007)
	EA.hy926, cell line	Parabolic flight, centrifuge	Differential regulation in gene expression of 320 genes was observed upon microgravity, partly up and partly downregulated. The cytoskeletal element β-tubulin underwent cytoplasmic rearrangement Hypergravity (1.8 g) reduced CARD8, NOS3, VASH1, SERPINH1, CAV2, ADAM19, TNFRSF12A, CD40, and ITGA6 mRNAs	Grosse et al. (2012)
	EA.hy926, cell line	RPM	After 5 days of simulated microgravity, genomic analysis revealed differentially regulated genes that are involved in the processes of signal transduction, are angiogenic factors, have a role in cell adhesion, membrane transport, or are enzymes involved in serine biosynthesis	Ma et al. (2013)
	EA.hy926, cell line	RPM	Cells responded with an increase in extracellular matrix proteins and changes in microtubules and intermediate filaments to microgravity. After 4 h of microgravity, and more pronounced after 72 h, morphological and biochemical signs of apoptosis were detected. Induction of apoptosis could be attenuated by the addition of VEGF	Infanger et al. (2006)

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Cell type/focus of investigation	Cell model	Research platform	Findings	Reference
	EA.hy926, cell line	Parabolic flight, centrifuge	Cells reacted with altered gene expression to microgravity. Regulated genes were involved in angiogenesis, cytoskeleton, extracellular matrix, cell cycle regulation, and apoptosis. Hypergravity in a centrifuge led to downregulation of Pan-actin, tubulin, and moesin proteins. Additionally many genes were up- or downregulated by hypergravity	Wehland et al. (2013)
	Porcine aortic ECs (PAECs)	RPM	Microgravity for 72 h led to a decreased cell number, an upregulation in the expression of proapoptotic genes, and a decrease in the expression of antiapoptotic and proliferative/survival genes. Changes of nucleus shape and dissolution of intracellular organelles was assessed by autofluorescence analysis. Cells lost their ability to respond to angiogenic stimuli	Morbidelli et al. (2005)
	Murine lung capillary endothelial cells (1G11 cells)	RWV bioreactor	Microgravity led to reversible inhibition of cell growth. After 72 h, cells exhibited an upregulation of p21, a decreased synthesis of IL-6, and an increased amount of NO	Cotrupi et al. (2005)
	Human pulmonary microvascular endothelial cells	Clinorotation (2D)	72 h of clinorotation led to a disappearance of desmosome-like junctions and induction of apoptosis (TUNEL-staining, upregulation of BAX and caspase-3 genes, downregulation of Bcl-2 gene, increased protein expression of caspase-3 and caspase-9, decreased protein expression of PI3K and p-Akt). Disruption of actin filament integrity was observed	Kang et al. (2011)
	Bovine aortic ECs (BAEC)	Centrifuge	Exposure to discontinuous hypergravity leads to changes is integrin distribution, cytoskeletal network reorganization, downregulation of proapoptotic signals, and reduced expression of genes involved in inflammation and vasoconstriction	Morbidelli et al. (2009)

	Bovine aortic ECs (BAEC)	Centrifuge	In bovine aortic ECs (BAECs) centrifugation at 3 g led to a transient reorganization of actin fibers in 3 min which was abolished by inhibitors of Rho-kinase and tyrosine kinase. 3 g hypergravity for 1 or 2 h/day enhanced endothelial migration in 5 days	Koyama et al. (2009)
	Bovine aortic ECs (BAEC)	RWV bioreactor	Cells growing as a three-dimensional culture on microcarrier beads for up to 30 days responded to microgravity with enhanced NO production (dependent on the rotation speed). Furthermore a decreased permeability of the cell layer was observed, accompanied with increased expression of the junctional complex proteins ZO-1 and occludin, indicating an enhanced barrier function of the endothelial layer	Sanford et al. (2002)
	Coronary venular ECs	Centrifuge	5 periods of 10 min exposure to 10 g separated by 10 min of recovery at 1 g led to downregulation of proapoptotic genes FADD, Fas, and FAS-L; upregulation of NF-kB; upregulation of genes for cytoskeletal proteins β-actin, α-tubulin, and vimentin; and altered cytoskeletal organization in coronary venular ECs	Monici et al. (2006)

4.1 Monocyte/Macrophage System

Cytokine Secretion As one of the central effector functions of monocytic and macrophageal cells, the secretion of cytokines under the influence of gravitational changes was investigated in numerous studies. Monocytes and T cells in co-culture respond to T cell activation with normal secretion of interleukin (IL)-2 and IL-1, respectively, under real microgravity on a Biosatellite (Limouse et al. 1991). Likewise upon stimulation of peripheral blood monocytic cells (PBMCs) with anti-CD-3 (leading to cell-cell contact between T cells and monocytes), the level of synthesized IL-1 by monocytes stayed unaffected in clinorotation (Hashemi et al. 1999). In contrast, if T cells and monocytes are cultured and stimulated separately, IL-2 and IL-1 secretion was reduced in microgravity (Limouse et al. 1991). Likewise, during an experiment in Spacelab ConA-stimulated peripheral blood monocytes responded with a strong decrease in IL-1 secretion to weightlessness (Cogoli et al. 1993), and in an experiment on a space shuttle flight, the synthesis of IL-1 β by U937 cells was decreased compared to controls (Schmitt et al. 1996). But there are also reports about upregulation of IL-1 secretion upon microgravity exposure in in vitro experiments: in the murine bone marrow macrophage cell line B6MP102, the lipopolysaccharide (LPS)-induced secretion of IL-1 and tumor necrosis factor-α (TNF-α) was higher during spaceflight compared to controls on earth (Chapes et al. 1994). In an ex vivo experiment with peripheral monocytes from astronauts taken after 13–16 days of spaceflight, cells responded to LPS stimulation also with an increased expression of IL-1β (Crucian et al. 2011). In the same study the expression of other cytokines was assessed revealing a decreased expression of IL-6, TNF-α, and IL-10. Expression of IL-8 was found to be regulated in either direction, dependent on the space flight mission (Crucian et al. 2011). Similar results were obtained in a study using a ground-based model of microgravity; in murine macrophageal cells that were cultured in a 3D rotating wall vessel (RWV) for 2 days followed by stimulation with LPS/interferon(IFN)-γ and cultivated for another 2 days, the production of TNF-α, IL-6, and IL-12 was decreased significantly compared to 2D cultured cells (Hsieh et al. 2005). In mouse macrophages cultured for 24 h in a rotary cell culture system (RCCS), the expression of LPS-induced TNF-α was markedly decreased compared to 1 g culture. Phosphorylation of IKK and JNK and nuclear translocation of NF-kB (processes of LPS-induced intracellular signal transduction) as well as TNF-α mRNA stability were not altered upon microgravity, but microgravity led to an upregulation of heat shock factor-1 (HSF1), a repressor of TNF-α promoter (Wang et al. 2014). In a later study under the same conditions, increased levels of IL-6 and decreased levels of IL-12B were measured (Wang et al. 2015). Additionally an increased level of arginase mRNA and protein levels and enhanced expression of C/EBPβ (a transcription factor which is relevant for arginase transcription) were reported (Wang et al. 2015). Taken together the secretion of cytokines is sensitive to real and simulated microgravity, and the nature of the changes is highly dependent on the microenvironment surrounding the cells.

Intracellular Signal Transduction Protein kinase C (PKC) is a central element of many signal transduction cascades. Upon activation, PKC is translocated into different cellular compartments where it transmits the signal by phosphorylation

of other proteins. Several studies provide evidence that PKC signaling is sensitive to altered gravity conditions. In an experiment on a space shuttle flight, it was observed that the subcellular distribution of PKC in U937 was sensitive to gravitational changes: with increasing acceleration, the level of PKC in the nuclear fraction decreased, while the level of PKC in the cytosolic fraction increased (Schmitt et al. 1996). The translocation of PKC from the cytosol to the particulate fractions of U937 cells upon stimulation with phorbol ester happened with an altered kinetic during space flight as compared to controls on the ground. Additionally the binding of a phorbol ester to PKC occurred to an enhanced extend in-flight, while hypergravity (1.4 g) leads to a decreased level of binding (Hatton et al. 1999). Experiments investigating the influence of microgravity on different isoforms of PKC revealed that phorbol ester-induced translocation of PKC beta II, delta, and epsilon to the particulate fraction was decreased during spaceflight compared to 1 g and was increased by 1.4 g. These alterations of PKC signaling have been discussed to be involved in the various changes in gene expression and cell functions induced by altered gravitational conditions (Hatton et al. 2002). In LPS-stimulated peripheral blood monocytes, real microgravity led to an impairment of Jun-N-terminal kinase activation compared to on-board 1 g controls. In contrast, activation of p38 MAP kinase was not altered (Verhaar et al. 2014). In another study simulated microgravity in a RCCS for 24 h led to activation of p38 MAPK in primary mouse (Wang et al. 2015). Weightlessness during parabolic flight led to enhanced overall tyrosine phosphorylation and activation of c-jun in non-stimulated monocytic U937 cells but to decreased overall tyrosine phosphorylation and reduced activation of c-jun in phorbol myristate acetate (PMA)-stimulated monocytic U937 (Paulsen et al. 2010). Thus, the effects of gravitational changes seem to be specific with respect to different signaling pathways and the activation status of the cells.

Phagocytosis In monocytes isolated ex vivo from the blood of astronauts after the return from spaceflight, the capacity to phagocytose was investigated. Spaceflight of 5–11 days decreased the percentage of phagocytosing monocytes and their phagocytic index as measured by the ability to engulf bacteria (Kaur et al. 2005).

Cytoskeleton and Locomotion In many cell types changes in the cytoskeleton upon exposure to microgravity have been observed, for example, in T lymphocytes and osteoblasts (Schatten et al. 2001; Hughes-Fulford 1991). Likewise, gravisensitivity of the cytoskeleton could be shown in macrophageal cells: in the monocytic cell line J-111, the cytoskeletal proteins actin, tubulin, and vinculin were altered in simulated microgravity in a random positioning machine (RPM) (Meloni et al. 2006). This effect could be affirmed in real microgravity when in experiments on the ISS, the cytoskeletal architecture of J-111 monocytes appeared to be markedly disrupted, as the distribution of F-actin, β-tubulin, and vinculin structures were severely changed under microgravity. Additionally a quantitative analysis showed a significant reduction of F-actin fibers. Possibly as a consequence of this disruption of the cytoskeleton, the ability of the cells to migrate was markedly decreased in microgravity samples compared to 1 g in-flight and ground controls (Meloni et al. 2011). Other experimental results point to a gravisensitivity of the adhesion-associated molecules CD62L and intercellular adhesion molecule 1 (ICAM-1) (Crucian et al. 2011; Paulsen et al. 2015).

Oxidative Burst A central effector function of macrophages is the oxidative burst, the release of reactive oxygen species (ROS) that dispose pathogens. Phagocytes and the NADPH oxidase enzyme-triggered oxidative burst reactions are part of the ancient innate immune system and represent the most important barrier for microbes invading the body. In murine macrophageal cells that were cultured under simulated microgravity in a 3D RWV for 4 days and stimulated with LPS/IFN-γ on day 2, the production of nitric oxide (NO) was decreased by 65 % (Hsieh et al. 2005). Using an elegant in vitro experimental setup that enables live measurement of ROS release, it was shown that the release of ROS during the oxidative burst by NR8383 rat macrophages was strongly decreased during parabolic flight and 2D clinorotation and was increased in hypergravity (centrifuge). These effects happen rapidly after alterations of the gravitational condition and are reversible (Adrian et al. 2013). When NR8383 macrophages were exposed to simulated microgravity in a fast rotating clinostat and stimulated with zymosan, curdlan, or LPS, they produced significantly less ROS compared to the samples with normal gravity. Furthermore the phosphorylation of spleen tyrosine kinase (Syk), as an early signaling step required for ROS production, was decreased in the clinorotated samples. At the same time, a later step in ROS production, the translocation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) to the nucleus, was not altered by simulated microgravity (Brungs et al. 2015). The TRIPLE LUX A ISS experiment (uploaded with SpaceX CRS-6, BIOLAB/COLUMBUS) provided direct evidence that ROS release is highly sensitive to altered gravity, is adapting very fast to an altered gravitational environment, and reacts within a certain range of gravitational forces (Thiel and Ullrich 2015).

Differentiation/Bone Marrow Cell Phenotype One study investigated the effect of real microgravity on the differentiation process of macrophageal cells in bone marrow. In a subpopulation of murine bone marrow cells that contained very granular macrophage-like cells, 13 days of spaceflight led to decreased expression of Ly6C, c-Fos, CD44(high), and Ly6G and an increased expression of F4/80, suggesting that spaceflight leads to an enhanced differentiation compared to ground controls (Ortega et al. 2009). Another study revealed that the expression of genes involved in the differentiation process of monocytes into macrophages is altered by real microgravity (Hughes-Fulford et al. 2008).

Proliferation and Cell Cycle Control Monocytic cells react to ground-based simulated microgravity with decreased proliferation and changes in cell cycle control: a decrease of proliferation was shown in U937 in RWV bioreactor, accompanied by a decreased level of cdc25B (Maier 2006; Cotrupi and Maier 2004). THP-1 cells that were cultured in a RCCS for 24 h also proliferated less than controls. Additionally the percentage of cells in the G0-Gl phase increased, and an inhibition of LPS-induced expression of tissue factor mRNA, a phosphatase involved in cell cycle control, was observed (Yu et al. 2011).

4.2 Natural Killer Cells

Natural killer (NK) cells belong to the cytotoxic lymphocytes and constitute an important link between the adaptive and the innate immune systems. They eliminate cells that are infected by viruses and cells that show tumorous features and thus play an important role in the control of inflammatory processes and tumor surveillance.

Early investigations of the effect of spaceflight on NK cells have revealed that after a 7-day space mission, the NK cells of astronauts displayed decreased killer activity and decreased inducible interferon production on the first day after return from the space mission (Talas et al. 1983). Similar results were obtained with NK cells from an astronaut after a 21-day mission; the cytotoxicity, the ability to bind and lyse target cells, and the percentage of NK cells were decreased after spaceflight (Konstantinova et al. 1995). Decreased numbers of NK cells were confirmed in further missions, and a dependence on the mission duration was suggested. After a 9-day mission, the number of NK cells was decreased, while it was unchanged after a 16-day mission (Stowe et al. 2003). In another study the percentages of NK cells were unchanged after space flight in four space shuttle missions that lasted 10–18 days (Crucian et al. 2000).

Subsequent in vitro experiments with NK cells exposed to real or simulated microgravity lead to partly conflicting results. In studies by Buravkova et al., NK cell exposure to real microgravity during a space mission and to simulated microgravity induced by clinorotation did not result in any alterations of cytotoxic activity toward target cells. Also the interferon production of NK cells upon binding of target cells in real microgravity did not differ from that of ground controls (Buravkova et al. 2004). The authors reason that immune cells do not lose their ability to bind to, recognize, and destroy target cells in vitro in microgravity (Buravkova et al. 2005). In contrast, evidence of a sensitivity of NK cells to microgravity in vitro was found in experiments using the RWV bioreactor as source of simulated microgravity. In these experiments IL-2 induced NK activity and lymphokine-activated killing of PBMCs were not different in cells under simulated microgravity and 1 g controls, but the upregulation of IL-2 receptor α chain (CD25) was decreased under simulated microgravity conditions. Furthermore, the secretion of the secondary cytokines IFN-γ, IL-1 β, and TNF-α was reduced. This suggests that in NK cells IL-2 pathways are differentially regulated in RWV-induced microgravity (Licato and Grimm 1999).

In 2013 Li et al. found that the cytotoxicity of NK cells was decreased upon exposure to simulated microgravity in a 2-D RWV. This effects could be counteracted by (IL)-15 alone or in combination with IL-12. Simulated microgravity led to increased levels of apoptosis and necrosis, and the expression of interferon (IFN)-γ and perforin was decreased. Among the surface receptors of the NK cells, NKG2A and NKG2D were expressed at a reduced level after exposure to simulated microgravity, while expression of NKp30 and NKp44 was not altered (Li et al. 2013).

4.3 Neutrophil Granulocytes/Polymorphonuclear Leukocytes

As the "first-line of defense," granulocytes protect the body from invading pathogens. They bind microorganisms, internalize them, and neutralize them with ROS in the phagosome, or they secrete ROS to kill pathogens extracellularly.

Several studies have shown that the number of neutrophil granulocytes is strongly increased in the blood of astronauts that have attended space flight for 5–16 days (Stowe et al. 1999, 2003; Kaur et al. 2004). The same effect has been observed in the blood of volunteers that were exposed to short-term microgravity during parabolic flight (Kaufmann et al. 2009).

Additionally to the number, also some effector functions of granulocytes have been reported to be affected by microgravity: in an in vitro experiment with murine *Propionibacterium acnes*-induced peritoneal inflammatory cells, the superoxide-anion production was found to be increased fourfold by microgravity during parabolic flight (Fleming et al. 1991). PMNs from the blood of individuals that had undergone parabolic flight showed an increased capability to produce hydrogen peroxide (H_2O_2) upon stimulation with N-formyl-methionyl-leucyl-phenylalanine (fMLP), fMLP and TNF-α, calcium ionophore (A23187), and PMA. Nevertheless, the spontaneous production of (H_2O_2) was not altered in that experiment (Kaufmann et al. 2009). Analysis of the regulatory mechanism of H_2O_2 release revealed that after parabolic flight, adenosine was more effective in controlling the release of cytotoxic H_2O_2 by primed PMNs and that this was due to an upregulation of the adenosine A2(A) receptor function (Kaufmann et al. 2011). After 8–11 days of spaceflight, granulocytes from the blood of astronauts reacted with a tenfold decrease in an optimal dose response chemotactic assay, indicating an enhanced chemotactic activity (Stowe et al. 1999).

Data regarding the effect of microgravity on further effector functions is scarce. In PMNs from blood of participants of parabolic flight, no differences in the ability to adhere and to phagocyte were detected (Kaufmann et al. 2009).

4.4 Dendritic Cells

Effects of microgravity on dendritic cells are largely unexplored. One study revealed that simulated microgravity affects the in vitro generation of dendritic cells: dendritic cells were generated from CD34+ progenitor cells from peripheral human blood in either a RCCS or in a static culture. Dendritic cell from the RCCS were less in number compared to the static culture; furthermore they had decreased capability for phagocytosis and a decreased density of HLA-DR on their surface and were less effective in antigen-induced responses (Savary et al. 2001).

4.5 Endothelial Cells

The effects of altered gravity on endothelial cells (ECs) are a broad field of research as endothelial functions are severely involved in cardiovascular homeostasis which is disturbed during spaceflight (Convertino 2009). Studies of the effect of altered gravity on many functions of ECs have been conducted. Here we will focus on the properties of ECs which are most directly connected to immunity, namely, the adhesive interaction with leukocytes, the cellular junctions and barrier function, the secretion of soluble substances relevant for the immune response, and gene expression. Additionally, the effects of altered gravity on the cytoskeleton of ECs are addressed. Integrity of the cytoskeleton is a prerequisite for almost all cellular functions as the cytoskeletal network is required not only to sustain the cellular morphology but also as a scaffold for transport of molecules inside the cell, for signaling processes, and for adhesion and migration.

Effects of altered gravity on proliferation, migration, and apoptosis are reviewed elsewhere (Maier et al. 2015).

Adhesion Adhesion of lymphocytes to the endothelium is a central step in immune reaction against intruding pathogens. During inflammation, adhesion is enabled by adjustment of the surface expression of adhesion molecules. Many reports describe the expression of adhesion molecules under altered gravity, while in only a few studies, adhesion could be examined on the functional level.

The data on the influence of simulated microgravity on human umbilical vein endothelial cells (HUVECs) are not very consistent. Grenon et al. found out that the gene expression and the surface presence of ICAM-1, VCAM-1, and e-selectin were significantly decreased after 24 or 48 h mechanical unloading in a RWV. These effects were reversible by addition of mechanical loading during the mechanical unload period (Grenon et al. 2013). In contrast, in a study using the RCCS (24 h), TNF-α-activated HUVECs reacted with significantly increased surface expression of ICAM-1 compared to 1 g controls. ICAM-1 mRNA expression was enhanced compared to the controls after 30 min and 1 h, while it was equal to the control after 24 h. Similar results were obtained for VCAM-1 (Zhang et al. 2010). The effect of microgravity seems to be dependent on the activation state of the cells and can be specific for different adhesion molecules as shown by Buravkova et al: in TNF-α stimulated HUVEC cells exposure to clinorotation for 18 h led to an increase of ICAM-1 expression but a decrease of e-selectin and VCAM-1. In non-stimulated cells exposure to microgravity also enhanced the expression of ICAM-1 but had no effect on e-selectin and VCAM-1 (Buravkova et al. 2005). In the same experiment functional investigation of the adhesion of lymphocytes to endothelial cells revealed that in a co-culture, the adhesion of phorbol ester-stimulated lymphocytes to HUVECs was enhanced by 18 h of clinorotation, while the adhesion of non-stimulated lymphocytes was not altered or was even slightly lower (Buravkova et al. 2005).

In an experiment in real microgravity on the ISS (8 days), HUVECs reacted with enhanced levels of IL-6, sICAM-1, and e-selectin in the culture supernatant and enhanced mRNA expression of IL-6, ICAM, and VCAM-1 indicating endothelial activation (Muid et al. 2010). During parabolic flight 20 s of real microgravity leads to an upregulation of ICAM-1 expression in HUVECs (Zhang et al. 2010). Wide-ranging genomic studies confirmed the regulation of genes involved in adhesion after 10 days of spaceflight in HUVECs (Versari et al. 2013) and after 5 days in a RPM in EA.hy926 (Ma et al. 2013).

Taken together, both promoting and decreasing effects of microgravity on adhesion molecules are found, making it difficult to predict the effect on adhesion. Nevertheless, the only functional study shows enhanced adhesion under microgravity conditions.

One study suggests that also hypergravity impacts adhesion properties of ECs: exposure of bovine aortic endothelial cells (BAECs) to discontinuous hypergravity (5×10 min at $10 \times g$ separated by 10 min at $1 \times g$) in a centrifuge leads to changes in integrin distribution, while adhesion itself was not affected (Morbidelli et al. 2009).

Cellular Junctions and Barrier Function Endothelial layers build a selective barrier for molecules, particles, and cells. Tight junction complexes between the cells of an endothelial layer strongly influence vascular permeability and leukocyte extravasation (Wallez and Huber 2008; Aghajanian et al. 2008). Unfortunately data is rare on the influence of altered gravity on the cellular junctions and the permeability of endothelial layers.

Sanford et al. investigated the functionality of the endothelial barrier in BAECs growing as a three-dimensional culture on microcarrier beads. Simulated microgravity in a RWV for up to 30 days led to a decreased permeability of the cell layer as assessed by measuring the transendothelial passage of particles. In accordance with this, the expression of ZO-1 and occludin, two junctional complex proteins that are located at tight junctions, was increased. These effects indicate an enhanced barrier function (Sanford et al. 2002). Kang et al. observed disappearance of junctions between pulmonary microvascular ECs upon 72 h of clinorotation, which would theoretically lead to decreased barrier function. But the disappearance of junctions was accompanied by the induction of apoptosis and might not be happening in viable ECs (Kang et al. 2011).

Release of Nitric Oxide (NO) NO is produced by ECs and immune cells. Additionally to its function as a toxic defense molecule, NO acts as a mediator of inflammatory responses. In NK cells, it is necessary for the cytotoxic activity and for responsiveness to IL-12 (Bogdan et al. 2000), and it influences the functional activity of other cell types including macrophages, neutrophils, and mast cells (Coleman 2001). NO production by ECs was repeatedly shown to be upregulated in HUVEC under simulated microgravity in a RWV and in a RPM (Versari et al. 2007; Carlsson et al. 2002). This upregulation was confirmed in two other types, BAECs and primary murine lung capillary ECs, which were

incubated in a RWV for several days (Sanford et al. 2002; Cotrupi et al. 2005). Nevertheless in a spaceflight experiment, NO production by HUVECs was found to be not altered after 10 days of microgravity (Versari et al. 2013). Interestingly hypergravity seems to cause also an upregulation of NO synthesis, as shown in two independent experiments with HUVECs: 5 g (MidiCAR) for 24 and 48 h and 3 g for 48 h led to an enhanced production of NO (Versari et al. 2007; Spisni et al. 2003).

Cytokines as Mediators of Inflammation Many studies document a downregulation of pro-inflammatory cytokines upon simulated microgravity. Incubation in a RWV for 24 or 48 h led to significantly decreased expression of IL-6 and TNF-α gene expression in HUVEC cells. The effects were reversible by addition of mechanical loading during the mechanical unloading period (Grenon et al. 2013). In an experiment using the same microgravity platform and the same cell type enhanced expression of heat shock protein 70, and a decreased level of IL-1 α was observed (Carlsson et al. 2003). Decreased synthesis of IL-6 was also observed in primary murine lung capillary ECs after 72 h in a RWV bioreactor (Cotrupi et al. 2005). In HUVECs a 2-D proteome analysis of cellular secretome revealed inhibited secretion of IL-1α and IL-8, while RANTES and eotaxin (leukocyte recruitment) secretion was increased after 96 h in a random positioning machine (Griffoni et al. 2011). In contrast to these rather anti-inflammatory effects, in real microgravity during a 10-day spaceflight, secretion of IL-1α and IL-1β was enhanced in HUVECs (Versari et al. 2013). In a study investigating other soluble factors, secretion of neurotrophic factor, ET-1, tissue factor, and VEGF was shown to be decreased after 10 days in a RPM in EA.hy926 cells (Infanger et al. 2007).

Gene Expression The influence of altered gravity on ECs becomes apparent also in the modulation of gene expression. Such alterations were observed in EA.hy926 cells as an effect of 5 days of simulated microgravity in a RPM and applied to genes that encode for angiogenic factors, enzymes that have a role in serine biosynthesis or are involved in the processes of signal transduction, cell adhesion, or membrane transport (Ma et al. 2013). In the same cell type, real microgravity during parabolic flight leads to altered expression of genes involved in angiogenesis, cytoskeleton, extracellular matrix, cell cycle regulation, and apoptosis (Wehland et al. 2013). In HUVECs, 10 days of spaceflight resulted in a significant modulation of genes that are involved in oxidative phosphorylation, cell adhesion, cell cycle, apoptosis, and stress response as compared to 1 g ground controls (Versari et al. 2013). Effects on gene expression were also observed upon hypergravity: exposure of BAECs to discontinuous hypergravity leads to a decrease in the expression of genes involved in inflammation and vasoconstriction (Morbidelli et al. 2009). In EA.hy926 cells hypergravity provided by a centrifuge led to reduced levels of CARD8, NOS3, VASH1, SERPINH1, CAV2, ADAM19, TNFRSF12A, CD40, and ITGA6 mRNAs (Grosse et al. 2012). In a similar study also up- or downregulation of numerous genes was shown (Wehland et al. 2013).

Cytoskeleton Today it is well accepted that the cytoskeleton is central to mechanosensation of physical parameters such as pressure and shear stress, allowing the cell to conduct physical into biochemical signals. It is also discussed to be the primary gravisensitive structure of mammalian cells.

In several independent studies, the effect of simulated microgravity provided by a RWV on HUVECs was assessed. Simulated microgravity led to remodeling of the actin cytoskeleton and, after several days, to a decreased amount of actin protein (Carlsson et al. 2003), Versari 2007 (Versari et al. 2007). In similar experimental setups, the disorganization of the F-actin fibers after 24 and 48 h simulated microgravity could be specified as perinuclear clustering (Grenon et al. 2013), and concomitant changes in clustering of ICAM were observed (Zhang et al. 2010). Griffoni et al. could show with a 2-D proteome analysis of the secretome that after 96 h in a RPM, the secretion of proteins relevant for the regulation of cytoskeleton assembly was altered (Griffoni et al. 2011). Experiments with EA.hy926 cells brought similar results: simulated microgravity in a RPM led to cytoskeletal alterations including changes in α- and β-tubulins, F-actin fibers, microtubules, and intermediate filaments (Infanger et al. 2006, 2007). After 72 h clinorotation, disruption of actin filament integrity was observed in human pulmonary microvascular ECs (Kang et al. 2011).

In only a few studies, the effect of real microgravity on the cytoskeletal architecture was investigated: during parabolic flight, β-tubulin underwent rearrangement and accumulated around the nucleus in EA.hy926 cells (Grosse et al. 2012). After 12 days of spaceflight, cytoskeletal lesions in HUVECs were observed. In the same study readapted cells which were cultivated after retrieval showed cytoskeletal changes that persisted for up to nine passages (Kapitonova et al. 2012).

Less data exists on the effects of hypergravity on the cytoskeleton of ECs. Nevertheless strong effects have been shown which can occur within minutes, and interestingly they are similar to the ones induced by microgravity: after long-term hypergravity of 96 h (3.5 g) in a MidiCAR centrifuge, actin fibers of HUVECs exhibited an altered distribution and tended to gather around the nucleus (Versari et al. 2007). Short-term hypergravity in BAECs (centrifugation at 3 g for 3 min) led to a transient reorganization of actin fibers mediated by RhoA activation and FAK phosphorylation (Koyama et al. 2009).

Exposure of the same cell type to discontinuous hypergravity of 5×10 min at $10 \times g$ separated by 10 min at $1 \times g$ also led to cytoskeletal network reorganization (Morbidelli et al. 2009). The same experiment was performed with coronary venular ECs as an EC cell type from the venular part of the vascular system. In this cell type also cytoskeletal network reorganization was seen, additionally upregulation of genes for the cytoskeletal proteins β-actin, α-tubulin, and vimentin was found (Monici et al. 2006). In EA.hy926 cells hypergravity led to downregulation of Pan-actin, tubulin, and moesin protein (Wehland et al. 2013).

Taken together effects of altered gravity on the cytoskeleton have been observed at the levels of fibers, proteins, regulators, and genes. Although the observations might not always be consistent, the fact that effects have been observed throughout

all used experimental platforms and investigated cell types leads to the assumption that the cytoskeleton is highly sensitive to altered gravity-induced changes.

4.6 Summary

In numerous in vitro and in vivo studies, strong and specific effects of micro- and hypergravity on cells of the immune system and on endothelial cells were revealed. Among the cells of innate immunity, the monocyte/macrophage system is the best studied. An altered gravitational environment has effects on cytokine secretion, intracellular signal transduction processes, phagocytosis, cell migration, cell differentiation, and cell proliferation in the monocyte/macrophage system. Some studies investigated the effect of altered gravity on NK cells, granulocytes, and dendritic cells and reported evidences of altered NK cell activity, cytokine secretion, apoptosis, expression of surface receptors, and the release of hydrogen peroxide. Endothelial cells have well-known mechanosensory properties and gravity-sensitive effects, such as different regulations of adhesion molecules, increased release of nitric oxide, and downregulation of pro-inflammatory cytokines, and different profiles of gene expression have been reported. Therefore, the gravitational environment influences not only basal cellular processes such as cell cycle control but also specific effector functions such as cytokine secretion, oxidative burst, and surface receptor patterns. In general, for all cell systems investigated, there is a lack of functional in vitro studies which could significantly contribute to integrate the knowledge about different effects at the cellular and molecular level. Finally, rearrangement and reorganization of cytoskeletal structures were found in lymphocytes, in macrophages, and in dendritic cells throughout different microgravity platforms. These cytoskeletal changes could contribute to all kinds of pathological conditions observed during altered gravity. Moreover, as the cytoskeleton transduces mechanical stimuli into biochemical signals, it is considered as the primary gravity-responsive element in mammalian cells.

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