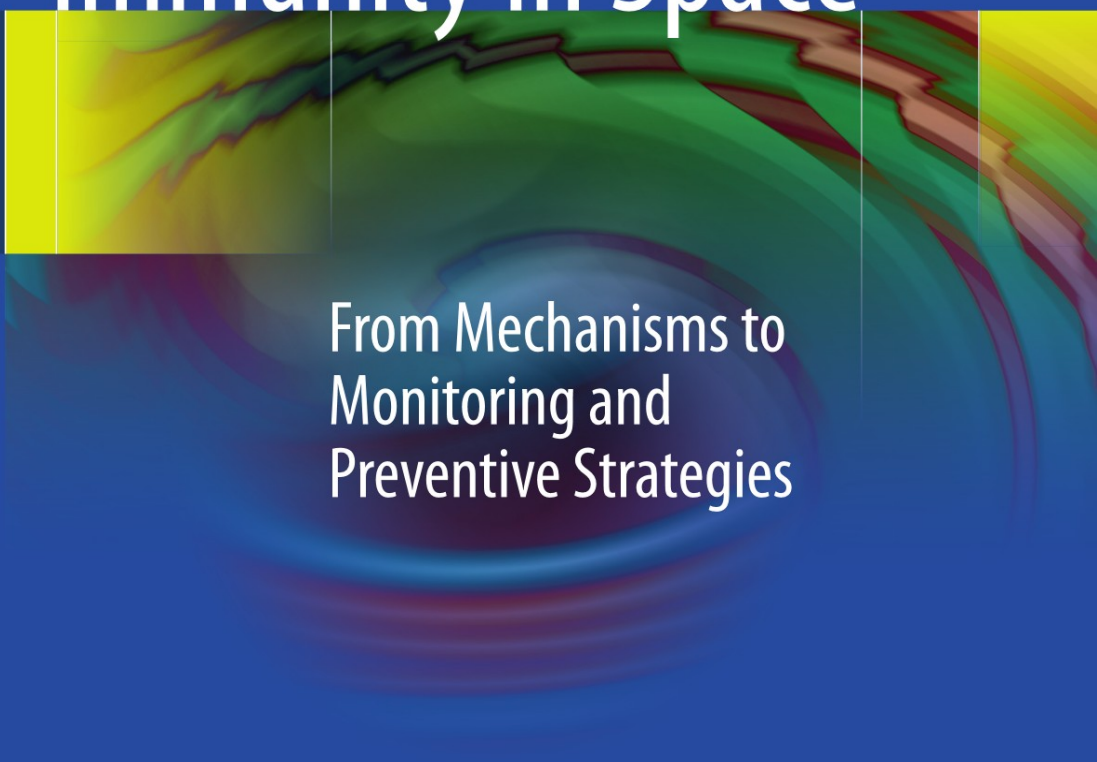
Alexander Choukèr *Editor*

Stress Challenges and Immunity in Space

From Mechanisms to Monitoring and Preventive Strategies



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To my wife Martina
To my children Marie-Thérèse, Maxime, Émile and Alphonse

Alexander Choukèr

Preface

"Stress Challenges and Immunity in Space" although seemingly specific in its title is broad in nature. The field of stress research is inherently multidisciplinary and complex as stress can arise from an almost limitless combination of situations and factors, and has the potential to influence all organ systems, either directly or indirectly. As a result, in approaching immune system changes during spaceflight, a highly unusual condition of life with a vast array of stressors and interactions, an integrative and holistic pathway is needed. Yet biological research in space is inherently limited in scope and detail. And therefore the question arises as to how to obtain sufficient detail and understanding to ensure the safety of our astronauts/cosmonauts.

This book is an attempt to approach this issue. It begins with a brief introduction to stress, describes the general interactions between stress, the central nervous system, and immunity; summarizes the current state of research with regard to immunity during spaceflight; and finally concludes with the latest technology and approaches to stress and immune monitoring, therapeutics, and future research platforms. The aim is not only to provide the current state of the art but also to serve as an impetus and drive for new research, which will eventually help mitigate the risks of voyage far beyond Earth. Furthermore, knowledge gained will help humans adapt to many extreme conditions of life, such as the critically ill, shift-workers, miners, Antarctic expedition crews, submariners, and more.

The participation of authors and expert scientists spanning a number of fields both from spaceflight and non-spaceflight research is a step toward an integrative and holistic approach, from basic science to applied science to technology. However, integrative and holistic implies that the current knowledge and views as presented are far from complete or comprehensive and by default are open to future discoveries and interpretations.

There, therefore, will be *space* to continue this approach. This book will hopefully serve as a starting point for a more integrative approach to research in the field of stress and immunity.

Munich, Germany — Alexander Choukèr

Acknowledgments

The support of many colleagues, partners, and international collaborators is much acknowledged, as without their support this book project would have not been completed. All contributing authors to this book deserve my highest appreciation for their work and for the extremely positive and kind collaboration during the preparative and review periods. The continuous input from all members of the ESA Topical Team "Stress and Immunity," their constructive criticism, and advice have been major sources of inspiration and the cornerstone for the realization of this book.

Special thanks go to Prof. Dr. Sarah Baatout and to Prof. Dr. Manfred Thiel for helping to generate the necessary momentum during the kick-off phase; and to Dr. Ines Kaufmann, Dr. Alex P. Salam, Sandra Matzel, Dr. Andrew Dobney, Marion Hörl, and to Dr. Chris Choukèr who were extremely supportive in the finalization period. This project would also not have been possible without the generous institutional support from the Department of Anaesthesiology at the Ludwig-Maximilians-Universität, and I want to especially thank the director Prof. Dr. Bernhard Zwissler for providing full encouragement and support at all times during this process.

My appreciation also goes to the scientists, doctors, and operators working in space, in space analogues, and extreme environments, as well as in clinical studies, and to the space agencies and funding institutions, who altogether have provided the intellectual input, experimental performance, and the financial means to realize the achievements presented in this volume. This acknowledgment extends to all participating volunteers and patients, as well as to the staff and students working in all the laboratories who provide critical and highly important contributions toward the further evolution of the field of stress and immune research in space, and on Earth.

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Part I

Prelude

Space Travel: A Personal View from Above

1

Thomas Reiter

Spaceflight has become an indispensable part of our daily life – it significantly contributes to the development of new technologies and applications. Furthermore, it helps to expand our knowledge about our own planet and our environment beyond the boundaries of our atmosphere. This applies especially to human space flight, for example on board the ISS, where research is performed in a wide spectrum of scientific disciplines.

Human space flight is also the source of great fascination. This constant, everyday curiosity drives us to continually expand our boundaries, to find answers to ever new questions. Curiosity is a deeply human quality, which has always played a central role in our development. Space is an excellent environment for research, which paves the way for solutions of earthly problems. Even in the medical field, for example in the understanding of stress and immune interactions, research in the space-environment provides unique possibilities.

The progression and further evolution of our technical and scientific knowledge isthe merit of generations of engineers and researchers, who have been working in the area of spaceflight and who will continue to push the limits of technology and science. In the next few decades, humans could be returning to the moon or travel to more distant destinations. More advanced spaceships will be needed, and a wide range of medical issues have to be solved. Specifically the impact of the space environment on the human body needs to be fully understood.

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Spaceflight is an interdisciplinary regime with a direct impact on science, technology and industrial capabilities. However, we should not loose sight of the human – the cultural – aspect of spaceflight. During training astronauts get prepared for every situation, for every off-nominal situation. Still, there is no preparation for this overwhelming view out of the window. This view gives a totally different perspective on our planet ‘from outside’. The sojourn in space is a totally new experience, no matter what you might have done before. After returning to earth, one wants to share this fantastic experience, the different views and insights with others. The various colours of our planet, cloud formations, the different colours of our atmosphere – a multitude of impressions that an astronaut registers, has to assimilate, and can never forget.

Space Travel: An Integrative View from the Scientists of the Topical Team "Stress and Immunity"

2

Sarah Baatout, Alexander Choukèr, Ines Kaufmann, Nicola Montano, Siegfried Praun, Dominique de Quervain, Benno Roozendaal, Gustav Schelling, and Manfred Thiel

For centuries, mankind has struggled to understand the profound complexity governing the principles of life and the universe. This quest has taken him on scientific journeys far and wide: from the exquisitely simple atomic structure of our DNA to the hellish and chaotic depths of our sun, the energy source for all life on Earth, and

The European Space Agency has supported the teaming up of international experts in "Topical Teams". Topical Teams are open structures lead by European researchers which should address a scientific field in which gravity and access to space or planetary bodies constitute important cornerstones. The founding members of the Topical Team "Stress and Immunity", as listed in alphabetical order as authors of this perlude, were significantly involved in the realization of this book, contributed to it and authored this chapter as a group.
This prelude was supported also by Alex P. Salam.

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beyond. Scientific, artistic, and social discoveries are what drive us as humans, and what distinguish us from all other species with which we share this planet. One of the fundamental questions that still troubles us is how life began on this planet and whether it exists elsewhere in the universe. This deep desire to understand and search for life has taken humans on exploratory journeys to the extremes of our planet: from the depths of our oceans to the heights of our mountains, and from the cold of Antarctica to the darkness of space. Fifty years ago, Yuri Gagarin marked a defining moment in the history of human exploration when he became the first human to escape the clutches of Earth's gravitational pull. Yet in 1960, before he launched into space, it was not even clear if humans could survive in a zero-gravity environment. At no stage in our evolution had we been prepared for such an environmental stress. From the moment life began in the "pre-biotic soup," some 3 billion years ago, all life on earth, Eukaryotes, Prokaryotes, and Archaea alike, have been shaped by the universal force of gravity. Within the space of a few minutes however, the most complex of these organisms, a human, Yuri Gagarin, seemingly "skipped" this evolutionary force and successfully coped with the absence of gravity. Since his historic 108-min voyage, others have survived for months not only in weightlessness, but also in extreme isolation and confinement, darkness, and danger. However, adapting to such hostile and unnatural conditions is not without any repercussions and is accompanied by adverse physiological and psychological effects, which, over the last decades, have been shown to disrupt almost all organ systems. Whilst our presence has extended beyond low Earth orbit to the moon, manned exploration beyond Earth's vicinity into the depths of our solar system requires a much more detailed understanding of the adaptation of human beings to extreme environments. Major questions remain: What are the principal and most important environmental and social threats to physical and mental health of crews during long-duration space flight missions, and how can we prevent and mitigate the adverse effects from adaptation to these threats?

It was Hans Selye who first used the term "stress" in the 1930s to describe how a biological system might adjust to the challenges and demands associated with major environmental changes (Selye 1936). He realized that when a complex organism is challenged by noxious conditions, the resulting symptoms are independent of the quality of the conditions, i.e., the qualitative end-result of different stressor types is the same. Rather, it is the quantitative effects that vary however. He also recognized that stressful conditions directly affect neural pathways, such as the autonomic nervous system, but also indirectly affect other organ systems, e.g., the immune system. The steps involved in the adaptation process to chronic stress are gradual and the biological system either builds up resistance to the stress and maintains a healthy physiological and psychological equilibrium, or succumbs to the stress, resulting in disequilibrium.

Stress research has expanded tremendously since then and Selye probably never imagined that it would transcend Earth's boundaries. Space flight is associated with a very distinct and unique combination of stressors: zero-gravity, radiation, altered microbial flora, isolation, confinement, altered day/night cycles and closed loop environments. Such stressors will be experienced in the extreme during inter-planetary travel. These combined and multi-factorial challenges affect many organ functions,

including immunity, and overall health. Moreover, in the case of the immune system for example, changes can influence other physiological systems, and even feedback with neural pathways in a bidirectional manner (Tracey 2009).

Although astronauts are exceptionally well selected, trained, and healthy individuals, some are now known to be particularly prone to health alterations during the course of space flight. When challenged by complex stressful conditions, e.g., space flight, individuals react differently and adjustment to the conditions can fail. The "milieu intérieur" (*Claude Bernard, 1813–1878*) is no longer able to maintain "coordinated physiological processes which maintain most of the steady states in the organism," as they "are so complex and so peculiar to living beings – involving, as they may, the brain and nerves, the heart, lungs, kidneys and spleen, all working cooperatively" (Cannon 1932). This concept of "homeostasis" is extended further by the notion of homeodynamics, i.e., "the stability of the internal milieu toward perturbation" (Lloyd et al. 2001).

Although studying specific cellular models and simple biological organisms under conditions of simulated weightlessness, increased radiation, or isolation and confinement can help unravel the neurophysiological consequences of standardized emotional and physiological strains, no organ, especially in the case of humans, can be considered as a stand-alone entity. For this reason, new integrative and holistic approaches to the understanding of stress responses and individual predispositions and reactions to stress have started to evolve. With the help of research on the International Space Station and in analogous conditions and environments – e.g., group isolation and confinement in chamber studies (e.g., MARS500) or field operational conditions (e.g., Antarctica or sub-aquatic habitats) – the impact of distinct emotional and physical stressors, or a combination thereof, can be investigated. This will eventually help with the understanding of the incremental effects of stress on organ allostasis, from an allostatic load to overload with subsequent exhaustion and failure to re-establish an appropriate equilibrium.

Because allostasis is a continuous and evolving process, efficient and simple tools to monitor physiological and behavioral adaptation processes and consequences during long-duration deep space missions are needed to enable early detection of disease and early implementation of appropriate countermeasures. Given that the reaction to stress can vary between individuals, how can we design strategies to meet the astronauts' individual needs under evolving and unpredictable conditions? This may prove very difficult and will require new technologies and devices. Should we select astronauts based on the presence of genetic characteristics that confer resistance to stress? The new technological tools of molecular biology, such as micro-arrays, will help to understand the genetic and epigenetic (e.g., DNA-methylation, post-transcriptional regulation) reasons for (mal) adaption, and therapeutic consequences. If genetic testing were to provide the potential to select and de-select candidates, this would have important ethical, social, and psychological implications. However, because "reading genes" is not equivalent to "understanding genes" and because human complexity goes beyond genetic heritage, identification of genetic polymorphisms that appear to correlate with a higher predisposition to physiological and behavioral stresses should not disqualify a potential space flight

candidate. Although polymorphisms in genes, for example, genes regulating sleep (Goel et al. 2009), or traumatic memory encoding, or DNA repair, may possibly lead to increased risk, individuals may have unidentified genetic resistance to other space-related stress factors, as well as behavioral resistance that may mitigate genetic risk. "The right stuff" seems very likely to be a very complex mix of gene–environment interactions. Given the ethical implications, the use of genetic analysis is *not* to define candidates who are suitable or not suitable for space flight, *but* rather to identify possible risks in order to personalize the frequency and mode of physiological and psychological assessments and countermeasures in space, and during rehabilitation upon return to Earth.

There is much left to qualify and quantify but with time we will refine the physiological, psychological, and pharmaceutical factors and interventions that will allow humans to travel inter-planetary distances. Along the way, these developments will not only benefit our space agencies but also wider society. Stress has the ability to alter the function of virtually every single organ system and cell type in the human body. The study of healthy humans experiencing high levels of stress in confinement and isolation, and in other space analogous environments, allows us to draw clear causal links between stress and physiological disequilibrium and disease. Understanding the interaction between stress and the human body and mind will lead to better healthcare not only for astronauts, but also for the vast majority of us who will never escape gravity's pull. Every single person on this planet experiences stress and no one is completely immune to its effects.

Acknowledgment The authors acknowledge the funding of this Topical Team by the European Space Agency (ESA) and express their thanks to Dr. Oliver Anger (ESA) for his support. The funding of the authors by national space agencies or other national or international funding institutions is acknowledged and specified in more detail in the authors' individual chapters

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The topics addressed in this prelude will be presented also in the parts II to VI of this volume. In addition, the following sources have been used to compile this prelude:

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Part II

"Stress and Immunity" - Research: A Link Between Space and Earth

What Is Stress?

3

Bruce S. McEwen and Ilia N. Karatsoreos

> 'I am stressed out' is non-accusatory, apolitical and detached. It is a good way to keep the peace and, at the same time, a low-cost way to complain.
>
> – America's Latest Export: A Stressed-Out World
> By Richard A. Shweder
> Published: January 26, 1997

3.1 Introduction

Stress is a word that is used throughout the world, and it has many meanings. There is "good stress" and "bad stress." Some would prefer to use "stress" to refer only to the experience and consequences of a situation when one is unable to cope physically or psychologically with the challenge (Cohen et al. 2007; Lazarus and Folkman 1984). Physiologically, cortisol and adrenalin are stress hormones and the fight or flight response is usually the focus of discussions of stress. But that is only part of the story. There are multiple biological mediators besides the adrenal stress hormones that are responsible for adaptation in situations that evoke the fight or flight response (McEwen and Stellar 1993; Sterling and Eyer 1988) and help us stay alive, but these same mediators also contribute to pathophysiology when overused and dysregulated, resulting in allostatic load and overload (McEwen 1998; McEwen and Wingfield 2003).

The brain is the central organ of stress and adaptation because it determines not only what is threatening, or at least different and potentially threatening, in a new

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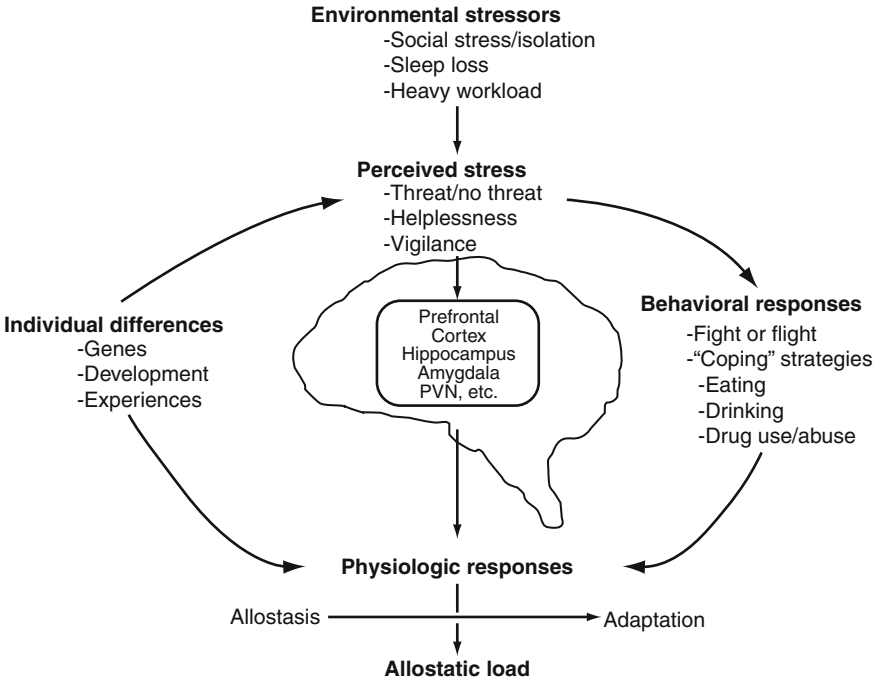

Fig. 3.1 The brain is the central organ of stress and adaptation and plays a major role in determining whether there is successful adaptation as well as cumulative wear and tear on the body and brain, i.e., allostatic load and overload. *PVN* paraventricular nucleus in the hypothalamus

situation, but also determines the physiological and behavioral responses (Fig. 3.1). Alterations in brain structure and function by experiences throughout life determine how each individual will respond to new events. But there are also important contributions from genes; individual life-style habits reflecting items, such as sleep quality and quantity; diet, exercise and substance abuse; adverse early life experiences that set life-long patterns of behavior and physiological reactivity; and exposure to toxic agents in the environment.

One purpose of this chapter is to describe the concepts of allostasis and allostatic load and overload as a way of making the discussion of the physiology and psychobiology of stress more precise and biologically based and related to life style and health-related behaviors, as well as the stressful experiences themselves. The other purpose of this chapter is to highlight ways in which the brain and its architecture play a key role in how individuals respond to new challenges. The relevance of the adaptation to stressors will be discussed in relation to the challenges of space flight and adaptation to microgravity providing further basis for understanding how the brain, immune system and health can be affected under such conditions.

Box 3.1: Levels of Stressful Experiences: Their Causes, Consequences and Why We Experience Them!

Positive Stress
- A personal challenge that has a satisfying outcome
- Result: Sense of mastery and control
- HEALTHY BRAIN ARCHITECTURE
- Good self-esteem, judgment and impulse control

Tolerable Stress
- Adverse life events buffered by supportive relationships
- Result: Coping and recovery
- HEALTHY BRAIN ARCHITECTURE
- Good self-esteem, judgment, and impulse control

Toxic Stress
- Unbuffered adverse events of greater duration and magnitude
- Result: Poor coping and compromised recovery
- Result: Increased life-long risk for physical and mental disorders
- COMPROMISED BRAIN ARCHITECTURE
- Dysregulated physiological systems

3.2 Types of Stress

This chapter will use the following classifications of types of stress: good stress, tolerable stress, and toxic stress (Box 3.1)*.

Good stress is a term used in popular language to refer to the experience of rising to a challenge, taking a risk and feeling rewarded by an often positive outcome. A related term is "eustress." Good self-esteem and good impulse control and decision-making capability, all functions of a healthy architecture of the brain, are important here. Even adverse outcomes can be "growth experiences" for individuals with such positive, adaptive characteristics.

"Tolerable stress" refers to those situations where bad things happen, but the individual with healthy brain architecture is able to cope, often with the aid of family, friends, and other individuals who provide support. Here, "distress" refers to the uncomfortable feeling related to the nature of the stressor and the degree to which the individual feels a lack of ability to influence or control the stressor (Lazarus and Folkman 1984).

Finally, "toxic stress" refers to the situation in which bad things happen to an individual who has limited support and who may also have brain architecture that reflects effects of adverse early life events that have impaired the development of good impulse control and judgment, and adequate self-esteem. Here, the degree

* See http://developingchild.harvard.edu/index.php/activities/council/ for the document 'The Science of Early Childhood Development: Closing the Gap Between What We Know and What We Do' as well as many other useful papers on the topic of brain development and stress

and/or duration of "distress" may be greater. With toxic stress, the inability to cope is likely to have adverse effects on behavior and physiology, and this will result in a higher degree of allostatic overload, as will be explained later in this chapter.

3.3 The Concepts of Allostasis and Allostatic Load and Overload

The body responds to many experiences by releasing chemical mediators – for example, catecholamines that increase heart rate and blood pressure. These mediators promote adaptation to simple acts like getting out of bed in the morning or climbing a flight of stairs or more complex acts, like giving a lecture or a musical performance. However, chronically increased heart rate and blood pressure can cause pathophysiological changes. For example, in the cardiovascular system, these changes can result, over time, in pathophysiological conditions like atherosclerosis, that can result in stroke and myocardial infarctions (Cohen et al. 2007).

Because these mediators are involved, paradoxically, in both protection and damage, and also because the word "stress" has ambiguities and connotations that interfere with its precise use, the term "allostasis" was introduced (Sterling and Eyer 1988) to refer to the active process by which the body responds to daily events and maintains homeostasis (allostasis literally means "achieving stability through change"). See Box 3.2 "Definitions." Because chronically increased allostasis can lead to pathophysiology, we introduced the terms "allostatic load or overload" (see distinction in Box 3.2 "Definitions" and below) to refer to the wear and tear that results from either too much stress or from inefficient management of allostasis, such as not turning off the response when it is no longer needed (McEwen 1998; McEwen and Stellar 1993; McEwen and Wingfield 2003).

Other forms of allostatic load/overload involve not shutting off the response efficiently, or not turning on an adequate response in the first place (McEwen 1998). Having many stressful events and many stress responses also contributes to "wear and tear" on the body and brain (McEwen 1998). Likewise, not habituating to the recurrence of the same stressor and thus dampening the allostatic response can also lead to overexposure of the brain and body to the mediators of allostasis (McEwen 1998).

Box 3.2: Definitions

Homeostasis is the stability of physiological systems that maintain life, used here to apply strictly to a limited number of systems such as pH, body temperature, glucose levels, and oxygen tension that are truly essential for life and are therefore maintained within a range optimal for each life history stage.

Allostasis is achieving stability through change, a process that supports homeostasis, i.e., those physiological parameters essential for life defined

above, as environments and/or life history stages change. This means that the "setpoints" and other boundaries of control must also change. There are primary mediators of allostasis such as, but not confined to, hormones of the hypothalamo–pituitary–adrenal (HPA) axis, catecholamines, and cytokines. Allostasis also clarifies an inherent ambiguity in the term "homeostasis" and distinguishes between the systems that are essential for life ("homeostasis") and those that maintain these systems in balance ("allostasis") as environment and life history stage change.

Allostatic state: The allostatic state refers to altered and sustained activity levels of the primary mediators, e.g., glucocorticoids, that integrate physiology and associated behaviors in response to changing environments and challenges such as social interactions, weather, disease, predators, pollution, etc. An allostatic state results in an imbalance of the primary mediators, reflecting excessive production of some and inadequate production of others (Koob and LeMoal 2001). Examples are hypertension, a perturbed cortisol rhythm in major depression or after chronic sleep deprivation, chronic elevation of inflammatory cytokines, and low cortisol that increases risk for autoimmune and inflammatory disorders. Allostatic states can be sustained for limited periods if food intake and/or stored energy such as fat can fuel homeostatic mechanisms. For example, bears and other hibernating animals preparing for the winter become hyperphagic as part of the normal life cycle and at a time (summer and early autumn) when food resources can sustain it.

Allostatic load and allostatic overload: The cumulative result of an allostatic state (e.g., a bear putting on fat for the winter) is allostatic load. It can be considered the result of the daily and seasonal routines; organisms have to obtain food and survive and extra energy needed to migrate, molt, breed, etc. Within limits, these are adaptive responses to seasonal and other demands. However, if one superimposes additional loads of unpredictable events in the environment such as disease, human disturbance, and social interactions, then allostatic load can increase dramatically. Type 1 allostatic overload occurs when energy demands exceed energy income as well as what can be mobilized from stores. Type 2 allostatic overload occurs if energy demands are not exceeded and the organism continues to take in or store as much or even more energy than it needs. This may be a result of stress-related food consumption, choice of a fat-rich diet, or metabolic imbalances (prediabetic state) that favors fat deposition. There are other cumulative changes in other systems, e.g., neuronal remodeling or loss in hippocampus, atherosclerotic plaques, left ventricular hypertrophy of the heart, glycosylated hemoglobin, and other proteins by advanced glycosylation end products as a measure of sustained hyperglycemia. High cholesterol with low HDL may also occur, and chronic pain and fatigue, e.g., in arthritis or psoriasis, may also occur associated with imbalance of immune mediators.

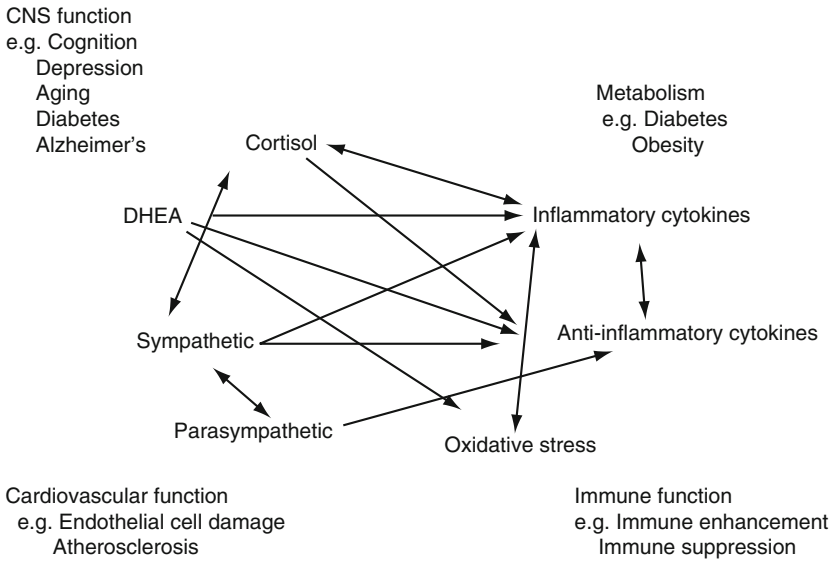

Fig. 3.2 Multiple interacting mediators and nonlinearity of interactions between them. *Arrows* represent direct and indirect regulatory influences of one mediator system upon the other systems. At the corners of the figure are listed some of the body systems that are concurrently affected by these mediators and their dysregulation (Reprinted from McEwen 2006 by permission). *DHEA* dehydroepiandrosterone

3.4 Multiple Interacting Mediators

Protection and damage are the two contrasting sides of the physiology involved in defending the body against the challenges of daily life, whether or not we call them "stressors." Besides adrenalin and noradrenalin, there are many mediators that participate in allostasis, and they are linked together in a network of regulation that is nonlinear, meaning that each mediator has the ability to regulate the activity of the other mediators, sometimes in a biphasic manner (Fig. 3.2). For example, glucocorticoids produced by the adrenal cortex in response to ACTH (adrenocorticotropic hormone) from the pituitary gland are the other major "stress hormone." Yet, pro- and anti-inflammatory cytokines are produced by many cells in the body, and they regulate each other and are, in turn, regulated by glucocorticoids and catecholamines. That is, whereas catecholamines can increase pro-inflammatory cytokine production (Bierhaus et al. 2003), glucocorticoids are known to inhibit this production (Sapolsky et al. 2000). Yet, there are exceptions – e.g., pro-inflammatory effects of glucocorticoids that depend on dose and cell or tissue type (Munhoz et al. 2010). The parasympathetic nervous system also plays an important regulatory role in this nonlinear network of allostasis, since it generally opposes the sympathetic nervous system and, for example, slows the heart, and it also has anti-inflammatory effects (Borovikova et al. 2000; Thayer and Lane 2000).

What this nonlinearity means is that when any one mediator is increased or decreased, there are compensatory changes in the other mediators that depend on time course and level of change of each of the mediators (McEwen 2006). Unfortunately, biomedical technology cannot yet measure all components of this system simultaneously and must rely on measurements of only a few of them in any one study, or their secondary consequences (McEwen and Seeman 1999). Yet the nonlinearity must be kept in mind in interpreting the results. One approach to "tap into" these mediators, and their surrogates, and obtain a broader picture of the network of allostasis is the "allostatic load battery" (McEwen and Seeman 1999; Seeman et al. 2010a, b).

A further, important aspect the mediators of allostasis is the biphasic nature of many of their effects, a concept embodied by the term "hormesis" (Calabrese 2008) and represented very clearly for cortisol (Joels 2006) and for pro- and anti-inflammatory cytokines, e.g., interleukin-6 (Campbell et al. 1993; Moidunny et al. 2010; Patterson 1992).

3.5 Stress in the Natural World

The operation of allostasis in the natural world provides some insight into how animals use this response to their own benefit or for the benefit of the species. As an example of allostasis, in springtime, a sudden snowstorm causes stress to birds and disrupts mating, and stress hormones are pivotal in directing the birds to suspend reproduction, to find a source of food and to relocate to a better mating site or at least to delay reproduction until the weather improves (Wingfield and Romero 2000). As an example of allostatic load, bears preparing to hibernate for the winter eat large quantities of food and put on body fat to act as an energy source during the winter (Nelson 1980). This accumulation of fat is used, then, to survive the winter and provide food for gestation of young. In contrast, the fat accumulation that occurs in bears that are captive in zoos and eating too much, partially out of boredom, while not exercising (McEwen and Wingfield 2003), is an example of "allostatic overload" referring to a more extreme condition that is associated with pathophysiology and is all-too-common in our own species.

Yet, allostatic overload can also have a useful purpose for the preservation of the species, such as in migrating salmon or the marsupial mouse, that die of excessive stress after mating – the stress, and allostatic load, being caused for salmon, in part, by the migration up the rapidly flowing rivers, but also because of physiological changes that represent accelerated aging and include suppression of the immune system (Gotz et al. 2005; Maule et al. 1989). One beneficial result of eliminating the adult salmon is freeing up food and other resources for the next generation. In the case of the marsupial mouse, it is only the males that die after mating, and the hypothesized mechanism is a response to mating that reduces the binding protein, CBG, for glucocorticoids and renders them much more active throughout the body, including likely suppressive actions on the immune defense system (Cockburn and Lee 1988).

3.6 Being "Stressed Out" and How It Affects the Way We Live

As noted by Shweder in the quote at the beginning of this chapter, while "stress" is an ambiguous term, the common experience of being "stressed out" has somewhat more specific meaning in terms of behavior and physiology and has, as its core, the elevation of some of the key systems that lead to allostatic overload – e.g., cortisol, sympathetic activity, and pro-inflammatory cytokines, with a decline in parasympathetic activity. When we are stressed out, we often feel frustrated, anxious, and even angry and we are likely to eat comfort foods, drink in excess of normal, smoke (if we are so inclined) and neglect regular moderate physical activity. We are also likely to sleep poorly. Indeed, poor or inadequate sleep is a frequent result of being "stressed out." Sleep deprivation produces an allostatic overload that can have deleterious consequences and a more detailed examination of this and circadian disruption via shift work and jet lag reveals how many aspects of health can be adversely affected.

3.7 Sleep Deprivation and Circadian Disruption as Sources of Allostatic Overload

Because the brain is the master regulator of the neuroendocrine, autonomic and immune systems, as well as behavior (McEwen 1998) (Fig. 3.1), alterations in brain function by chronic stress can have direct and indirect effects on cumulative allostatic load. One of the key systems in the brain and body that regulates homeostasis of these varied physiological and behavioral variables is the circadian system. Based in the suprachiasmatic nucleus (SCN) of the hypothalamus, the brain's clock controls rhythms in the rest of the brain and body through both neural and diffusible signals. Biological clocks at the molecular level have been detected in almost every body organ and tissue so far examined, and these clocks are synchronized by the SCN directly (by way of neural connections) or indirectly through hormonal signals (e.g., cortisol, melatonin) or behavioral outputs (e.g., feeding). The SCN also regulates the timing of sleep, and as such, sleep and circadian systems interact to regulate rest–activity cycles and keep an organism in synchrony with the external environment. As such, disruption of these key homeostatic systems could clearly contribute to allostatic overload.

Reduced sleep duration has been reported to be associated with increased body mass and obesity in the NHANES study (Gangwisch et al. 2005). Sleep restriction to 4 h of sleep per night increases blood pressure, decreases parasympathetic tone, increases evening cortisol and insulin levels, and promotes increased appetite, possibly through the elevation of ghrelin, a pro-appetitive hormone, along with decreased levels of leptin (Spiegel et al. 1999, 2004; Van Cauter et al. 1997). Moreover, pro-inflammatory cytokine levels are increased with sleep deprivation, along with decreased performance in tests of psychomotor vigilance, and this has been reported to result from a modest sleep restriction to 6 h per night (Vgontzas et al. 2004).

Circadian disruption has sometimes been overlooked as a separate yet related phenomenon to sleep deprivation. In modern industrialized societies, circadian disruption can be induced in numerous ways, the most common of which are shift work and jet lag. A longitudinal study in a cohort of nurses in night shift work found that exposure to night work can contribute to weight gain and obesity (Niedhammer et al. 1996). Moreover, alternating shift work is an independent risk factor for the development of obesity in a large longitudinal study of male Japanese shift workers (Suwazono et al. 2008). Numerous mouse models have also contributed to our understanding of the relationship between circadian disruption and metabolism: CLOCK mutant mice show altered basal metabolism and a tendency toward obesity and metabolic dysregulation (Turek et al. 2005), while normal C57Bl/6 mice housed in a disrupted 10 h light: 10 h dark cycle show accelerated weight gain and disruptions in metabolic hormones (Karatsoreos et al. 2011). Behaviorally, circadian disruption can contribute to cognitive impairments. In a study of long recovery vs. short recovery flight crews, it was found that short recovery crews had impaired performance in a psychomotor task, reacting more slowly, and with more errors when compared to a long recovery crew (Cho 2001).

3.8 The Brain as a Target of Stress and Allostatic Load and Overload

The brain is a target of stress and stress hormones, and the processes of allostasis and allostatic load and overload are exemplified by how different brain regions respond to acute and chronic stressors. Because the hippocampus was the first higher brain center that was recognized as a target of stress hormones, it has figured prominently in our understanding of how stress impacts brain structure and behavior, and will be explored first. Effects of stress on the amygdala and prefrontal cortex will then be summarized.

3.8.1 The Hippocampus

The hippocampus plays a key role in learning and remembering declarative and spatial information, as well as processing the contextual aspects of emotional events, and regulating visceral functions, including the HPA axis. The hippocampus, which is interconnected with the amygdala and prefrontal cortex (Petrovich et al. 2001), contains receptors for adrenal steroids, and for major metabolic hormones such as insulin, leptin, ghrelin, and insulin-like growth factor 1 (IGF-1), all of which have effects on the hippocampus (McEwen 2007). Specifically, these mediators can enhance cognitive processes, affect mood and motivation, and promote excitability and neuroprotection. Yet, these same mediators can have deleterious effects on the hippocampus under conditions associated with chronic stress and allostatic overload, the most extreme being seizures, stroke, and diabetes (Gold et al. 2007; McEwen 2007; Sapolsky 1992).

A number of animal models demonstrate that chronic stressful experiences (e.g., prolonged immobilization, housing in dominance hierarchies, early maternal separation) can remodel hippocampal neurons and result in changes in the morphology of the hippocampus. Within the hippocampus, input from the entorhinal cortex to the dentate gyrus is ramified by connections between the dentate gyrus and the CA3 pyramidal neurons. Hence, one granule neuron innervates, on average, 12 CA3 neurons, and each CA3 neuron innervates, on average, 50 other CA3 neurons via axon collaterals, as well as 25 inhibitory cells via other axon collaterals. The net result is a 600-fold amplification of excitation, as well as a 300-fold amplification of inhibition, that provides some degree of control of the system (McEwen 1999).

As to why this type of circuitry exists, the dentate gyrus-CA3 system is believed to play a role in the memory of event sequences, although long-term storage of memory occurs in other brain regions. But, because the DG-CA3 system is so delicately balanced in its function and vulnerability to damage, there is also adaptive structural plasticity; that is, CA3 pyramidal cells undergo a reversible remodeling of their dendrites in conditions such as hibernation and chronic stress. The role of this plasticity may be to protect against permanent damage (McEwen 1999, 2007).

Another type of structural plasticity involves replacement of neurons via neurogenesis. The sub-granular layer of the dentate gyrus contains cells that have some properties of astrocytes (e.g., expression of glial fibrillary acidic protein) and which give rise to granule neurons (Seri et al. 2001). After Bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU) administration to label DNA of dividing cells, these newly born cells appear as clusters in the inner part of the granule cell layer, where a substantial number, i.e., 5000–9000 per day, will subsequently differentiate into granule neurons within just 7 days (Cameron et al. 1993; Cameron and McKay 2001; Gould and Gross 2002). There are many hormonal, neurochemical, and behavioral modulators of neurogenesis and cell survival in the dentate gyrus, including estradiol, insulin-like growth factor 1 (IGF-1), antidepressants, voluntary exercise, and hippocampal-dependent learning (Duman et al. 2001; Gould et al. 1999; Trejo et al. 2001; van Praag et al. 1999). With respect to stress, certain types of acute stress and many chronic stressors suppress neurogenesis or cell survival in the dentate gyrus, and the mediators of these inhibitory effects include excitatory amino acids acting via *N*-methyl-D-aspartic acid (NMDA) receptors and endogenous opioids (for review, see McEwen 2007).

Another form of neuroplasticity is the remodeling of dendrites in the hippocampus. Chronic restraint stress causes retraction and simplification of dendrites in the CA3 region of the hippocampus (McEwen 1999). Such dendritic reorganization is found in both dominant and subordinate rats undergoing adaptation of psychosocial stress in the visible burrow system, which is independent of adrenal size (McKittrick et al. 2000). What this particular result emphasizes is that it is not adrenal size or presumed amount of physiological stress per se that determines dendritic remodeling, but a complex set of other interacting factors that modulate neuronal structure. Indeed, in species of mammals that hibernate, dendritic remodeling is a reversible process, and it occurs within hours of the onset of hibernation in European hamsters and ground squirrels (Magarinos et al. 2006; Popov et al. 1992). Moreover, it is

reversible within hours of wakening of the animals from torpor (Magarinos et al. 2006). This implies that reorganization of the cytoskeleton is taking place rapidly and reversibly and that changes in dendrite length and branching are not "damage" but a form of structural plasticity.

The plasticity of the hippocampus in animal models has fostered studies of the human hippocampus that have revealed functional and structural changes that are consistent with the animal model studies even though it is not possible to look at individual neurons in the living human brain (McEwen and Gianaros 2010). Functionally, spatial memories activate the human hippocampus, as in the London cab driver studies (Maguire et al. 1997). Further, intensive learning experiences are associated with increases in hippocampal volume, further suggesting dynamic experience-dependent neuroplasticity in the hippocampus (Draganski et al. 2006). Smaller hippocampal volume has been reported in Cushing's disease, recurrent major depression, Type 2 diabetes, posttraumatic stress disorder, a prolonged history of high perceived stress, chronic systemic inflammation, chronic jet lag, lack of regular exercise, and low self-esteem (McEwen and Gianaros 2010). With respect to exercise, there is evidence that physical fitness is associated with larger hippocampal volume (Erickson et al. 2009).

3.8.2 Amygdala

A critical function of the amygdala in stressor-related processing involves fear, anxiety, and aggression. Sensory input is relayed through thalamic and cortical-thalamic pathways to the basolateral area and then to the central nucleus. As a primary output nucleus, the central nucleus activates adrenalin and cortisol output and freezing behavior (LeDoux 2003). The central nucleus is also networked with cortical areas involved in stressor-related processing, including the anterior cingulate cortex, ventromedial prefrontal cortex, and orbital prefrontal cortex (McEwen and Gianaros 2010).

Chronic immobilization stress of the type that causes retraction of dendrites in the CA3 region of the hippocampus, produces dendritic growth in neurons in basolateral amygdala (Vyas et al. 2002). Moreover, chronic stress of this type not only impairs hippocampal-dependent cognitive function, but also enhances amygdala-dependent unlearned fear and fear conditioning; processes that are consistent with the opposite effects of stress on hippocampal and amygdala structure (Conrad et al. 1999; Wood et al. 2008).

Chronic stress also increases aggression between animals living in the same cage, and this is likely to reflect another aspect of hyperactivity of the amygdala (Wood et al. 2008). Moreover, chronic corticosterone treatment in drinking water produces an anxiogenic effect in mice (Ardayfio and Kim 2006; Karatsoreos et al. 2010), an effect that could be due to the glucocorticoid enhancement of CRF activity in the amygdala (Makino et al. 1994).

As is the case for the hippocampus, neuropsychological and imaging studies on the human amygdala have revealed biphasic changes in volume in acute versus

chronic depression (Frodl et al. 2003; Sheline et al. 1998), as well as increased functional activity (Drevets et al. 1992; Sheline et al. 2001). Moreover, functional amygdala reactivity to facial expressions is increased by sleep deprivation (Yoo et al. 2007), an early life history of lower socioeconomic status (Gianaros et al. 2009), and predisposition to cardiovascular disease (Gianaros et al. 2008).

3.8.3 Prefrontal Cortex

The prefrontal cortex is networked with the amygdala and exerts downstream control over amygdala activity, as well as midbrain and brainstem functions, including functions of control, as opposed to learned helplessness (Amat et al. 2005; McEwen and Gianaros 2010), and autonomic balance (Buchanan et al. 2010). Chronic stress also causes functional and structural changes in the medial prefrontal cortex, particularly in areas of anterior cingulate, prelimbic, infralimbic, and orbitofrontal regions. For example, chronic stress causes not only dendritic shortening in medial prefrontal cortex (Dias-Ferreira et al. 2009; Liston et al. 2006; Radley et al. 2004; Wellman 2001), but also produces dendritic growth in orbitofrontal cortex (Liston et al. 2006). Taken together with the differential effects of the same stressors on the hippocampus and amygdala, these actions of stress are reminiscent of recent work on experimenter versus self-administered morphine and amphetamine, in which different, and sometimes opposite, effects were seen on dendritic spine density in orbitofrontal cortex, medial prefrontal cortex, and hippocampus CA1 (Robinson and Kolb 1997). For example, amphetamine self-administration increases spine density on pyramidal neurons in the medial prefrontal cortex and decreases spine density on orbitofrontal pyramidal neurons (Crombag et al. 2005).

Behavioral correlates of CRS-induced remodeling in the prefrontal cortex include impairment in attention set shifting (Liston et al. 2006) and other tests of cognitive flexibility and decision making (Dias-Ferreira et al. 2009), possibly reflecting structural remodeling in the medial prefrontal cortex. In a circadian disruption paradigm which may represent a chronic low-level allostatic load, we have observed remodeling of neurons in the prelimbic region of the medial prefrontal cortex of mice which resemble those observed in chronic stress, including a loss of dendritic branching and reduction in length of the apical dendrites, though no decrease in spine density has been observed (Karatsoreos et al. 2011). Behaviorally, these circadian disrupted animals show normal acquisition of a spatial navigation task, but show impaired cognitive flexibility and increased error rates when made to switch their learning to a new location. This suggests that while chronic circadian disruption may not impair simple cognitive tasks, more complex tasks requiring flexibility may be impaired.

In the human prefrontal cortex, there are reported changes in functional connectivity in medical students under stress that relate to reduced performance on a test of cognitive flexibility like the attention set shifting study in rodents; these changes are reversible with vacation, supporting the concept of reversible neuroplasticity (Liston et al. 2009).

3.9 Acute Versus Chronic Stress

The essence of the concept of allostasis and allostatic load/overload is that the mediators of stress and adaptation have different effects in situations of acute stress versus chronic stress. We have discussed the effects of various types of stressors for the body as a whole and also for three key brain regions. One way of visualizing the range of effects of various stressful events for the hippocampus is as an inverted U in which acute levels of adrenal steroids acting together with excitatory amino acids and other intracellular mediators do the following things in space and time:

1. Low physiological levels acutely enhance excitability of neurons (Diamond et al. 1992; Pavlides et al. 1996) and enhance contextual fear memory (see also Chap. 5 in this volume).
2. Higher physiological and supraphysiological levels have the opposite effect and acutely suppress excitability (Diamond et al. 1992; Joels 2006; Pavlides et al. 1996).
3. Chronic stress or chronic elevation of glucocorticoids produces "adaptive plasticity" involving the changes in circuitry described in the previous section on the hippocampus.
4. Uncontrolled events such as head trauma, seizures, and stroke activate both adrenal steroids and excitatory amino acids in a way that leads to damage and neuronal loss (Sapolsky 1992).

Other physiological systems show differences in effects of acute versus chronic stress. Effects of stress on delayed-type hypersensitivity (DTH) in the immune system show that acute restraint stress enhances DTH whereas chronic restraint stress for 21 days suppresses it (Dhabhar and McEwen 1997, 1999). Moreover, the acute stress effect shows a dose–response relationship in that an acute restraint plus shaking of the animal produces a larger enhancement of DTH than acute restraint stress alone (Dhabhar and McEwen 1997, 1999). In the hippocampus, chronic restraint for 21d produced the remodeling of dendrites of CA3 neurons described, whereas lesser durations of chronic restraint stress did not cause such remodeling (Magarinos and McEwen 1995). Yet a shorter (10 days) period of a more intense stress, namely, chronic immobilization, caused CA3 dendrites to shrink (Vyas et al. 2002).

The essence of the difference between "tolerable" vs. "toxic" stress (Box 3.1) is the sense of control or lack thereof, as well as the quality and quantity of social support and integration (McEwen and Gianaros 2010). As noted earlier in this chapter, "tolerable stress" refers to those situations where bad things happen but the individual with health brain architecture is able to cope, often with the aid of family, friends, and other individuals who provide support. In contrast, "toxic stress" refers to the situation in which bad things happen to an individual who has limited support and who may also have brain architecture that reflects effects of adverse early life events that have led to impaired development of good impulse control and judgment and adequate self-esteem (Shonkoff et al. 2009) (http://developingchild.harvard.edu/initiatives/council/). Thus, with toxic stress, the inability to cope is likely to have adverse effects on behavior and physiology, and this will result in a higher degree of allostatic overload.

The sense of control has several important neurobiological ramifications. Learned helplessness is accompanied by a failure of top-down prefrontal cortical control of

midbrain serotonergic activity (Amat et al. 2005). Moreover, lack of control of tail shock stress in a classical eye blink conditioning paradigm revealed a stress effect that inhibited performance in females, but enhanced performance in stressed males (Wood and Shors 1998); giving the rats control of the tail shock stress abolished the stress effect on performance as well as the sex difference (Shors et al. 2007).

Lack of control is often exacerbated by, and related to, low self-esteem and low self-esteem has been linked to a smaller hippocampus and elevated cortisol secretion, with lack of habituation to repeated public speaking challenges (Kirschbaum et al. 1995; Pruessner et al. 1999, 2005). It remains to be seen what other aspects of brain function are altered under these conditions and whether interventions that improve an individual's self-esteem and locus of control can alter brain structure and function in measurable ways.

3.10 Space Flight, Microgravity, and Stress

Space flight, with reduced gravity and all the behavioral changes that are required of the astronaut, is very likely to change brain structure and function, as well as affecting hormone output, energy expenditure, cardiovascular, and autonomic and immune system function [see also Chaps. 5–13] (Blanc et al. 2001; Strollo et al. 1998; Tipton et al. 1996). The experience of microgravity, which increases glucocorticoid levels (Macho et al. 1993) has been shown to increase dendritic growth by acting through serum- and glucocorticoid-inducible kinase 1 (David et al. 2005). Seven days of microgravity has been shown to result in a loss of proteins in hippocampus (Sarkar et al. 2006). Further studies of brain regions involved in cognitive function and emotional control are warranted. This is especially true for prolonged space flight where neuroendocrine adaptations to shorter space missions may be diminished (Strollo et al. 1998; Tipton et al. 1996). Exposure to microgravity has been experimentally demonstrated to alter the length of the free-running (i.e., endogenous) period in insects (Hoban-Higgins et al. 2003), as well as altering the nycthermal distribution of energy expenditure in rats (Blanc et al. 2001). Importantly, circadian rhythms appear to function up to 90 days in space but weaken thereafter, along with disruptions in sleep (Monk et al. 2001). Consequences of circadian and sleep disruption such as those discussed earlier in this article must be undertaken to fully appreciate the metabolic, neurological, behavioral, and immune system consequences of weightlessness and prolonged space missions.

3.11 Conclusion: Toward a Better Understanding of Stress Effects on Brain and Immune, Metabolic, Cardiovascular, and Other Body Systems

Stress is a word that has communication value in our stressed-out world because it identifies a state-of-mind that is common among many people and provides an explanatory excuse for being harried and in a rush, as well as a means of generating

understanding and sympathy. This chapter has described a physiological and neurobiological foundation that should permit a better understanding of the consequences of acute and chronic stress on the body and brain; it has placed this foundation in the context of life-long influences of the social, as well as physical environments. The brain is the key to what these experiences do to the body because it determines threat and controllability and activates the behavioral and physiological responses, as well as the lifestyle choices and health-related behaviors that result from our experiences. We have seen that the brain is also a target of stress and changes in brain architecture do determine how the brain will respond.

The experiences of space flight are both physical and psychological stressors and likely sources of allostatic load and overload, particularly when they persist over weeks and months, and there is great need to better understand the ability of the body and brain to adapt to both the acute and chronic aspects of these experiences. The analysis provided in this chapter and in other chapters in this volume will help in this quest by directing attention to the role of the brain, its structural and functional plasticity, and its interaction with the immune system as well as metabolic, cardiovascular, and other body systems in relation to health and disease.

Moreover, the view on this interacting nature of the brain-dependent biological hormonal and neural mediators with other, direct gravitational, metabolic, microbial, or radiation-dependent consequences of the stressful living conditions in space will provide us a more complex insight into the adaptation of human organ-systems and especially of the immune system, which, as noted above, shows biphasic effects of the mediators of stress and adaptation. This is of importance in order to find the appropriate means to prevent the occurrence and/or the negative consequences of prolonged allostatic states and resulting allostatic overload that can result in disease, in space as well as on Earth.

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The Impact of Everyday Stressors on the Immune System and Health

4

Lisa M. Christian and Ronald Glaser

4.1 Stress and Inflammation

Much research linking stress and immune function has focused on inflammatory processes. Although inflammation is an essential immune response to infection or injury, exaggerated and/or chronic elevations in inflammatory proteins are detrimental to health. Indeed, inflammation contributes to risk and severity of chronic health conditions including cardiovascular disease, arthritis, diabetes, inflammatory bowel disease, periodontal disease, certain cancers, and age-related functional decline (Hamerman et al. 1999; Ershler and Keller 2000; Bruunsgaard et al. 2001; Black and Garbutt 2002; Ishihara and Hirano 2002). Via neuroendocrine pathways, psychological stress directly promotes elevations in circulating inflammatory proteins and primes the immune system to exhibit exaggerated inflammatory responses when faced with an immune challenge (e.g., exposure to an infectious agent).

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Caregiving provides an important model for assessing the effects of chronic stress on health; individuals who provide care for loved ones with chronic medical conditions, such as a spouse with dementia, commonly experience long-term stress characterized by significant life change and social isolation. We found that the chronic stress of caregiving exacerbated typical age-related increases in the proinflammatory cytokine interleukin(IL)-6; caregivers experienced fourfold greater increases in IL-6 in a 6-year longitudinal study compared to controls (Kiecolt-Glaser et al. 2003). Thus, stress accelerated this aspect of the aging process and increased risk for age-related diseases as already mentioned.

Proinflammatory cytokines also rise in response to acute stressors such as being told that one is laid off or having an argument. Indeed, relatively minor and brief laboratory stress tasks, such as public speaking, result in increases in circulating inflammatory markers (Steptoe et al. 2001; Brydon et al. 2004). The magnitude of inflammatory response seen following objective stressors is not necessarily predicted by subjective perceptions of stress (e.g., Brydon et al. 2004).

Importantly, repeated exposure to a stressor may not lead to habituation of inflammatory responses. For example, healthy middle-aged men were exposed to the same laboratory stressor involving public speaking three times with 1-week intervals between sessions. Although cortisol and blood pressure reactivity to the task habituated over time, stress-induced elevations in IL-6 were equivalent across visits (von Kanel et al. 2005). Thus, exposure to relatively minor but recurrent stress in daily life may contribute to morbidity and mortality by promoting inflammation.

In addition to directly promoting elevations in inflammatory markers, chronic stress and depressive symptoms can also "prime" the immune system to show an exaggerated inflammatory response upon exposure to psychological stressors and biological challenges (Anisman et al. 1999; Maes 1995; 1999; Glaser et al. 2003, Johnson et al. 2002; Maes et al. 2001; Stark et al. 2002; Avitsur et al. 2005; Quan et al. 2001; LeMay et al. 1990; Zhou et al. 1993). For example, rats exposed to repeated stressors mount exaggerated inflammatory responses upon exposure to an antigen in vivo and by cells stimulated in vitro (Johnson et al. 2002; Stark et al. 2002; Avitsur et al. 2005). Similarly, we have shown that, among older adults (Glaser et al. 2003) as well as healthy pregnant women (Christian et al. 2010), greater depressive symptoms predicted greater inflammatory responses to a challenge with an influenza virus vaccine.

In sum, stress as well as psychological distress (e.g., depressive symptoms) can promote inflammation directly, and prime the immune system to show an exaggerated inflammatory response when challenged. Via these pathways, exposure to chronic psychological stress as well as minor but repeated stressors may ultimately contribute to risk of a variety of health conditions for which inflammatory processes are implicated.

4.2 Stress and Wound Healing

The skin provides the body's first line of immune defense by providing a physical barrier against invasion by pathogens as well as antigens and limiting the movement of water in and out of the body (Elias 2005; Marks 2004). Thus, the integrity of the

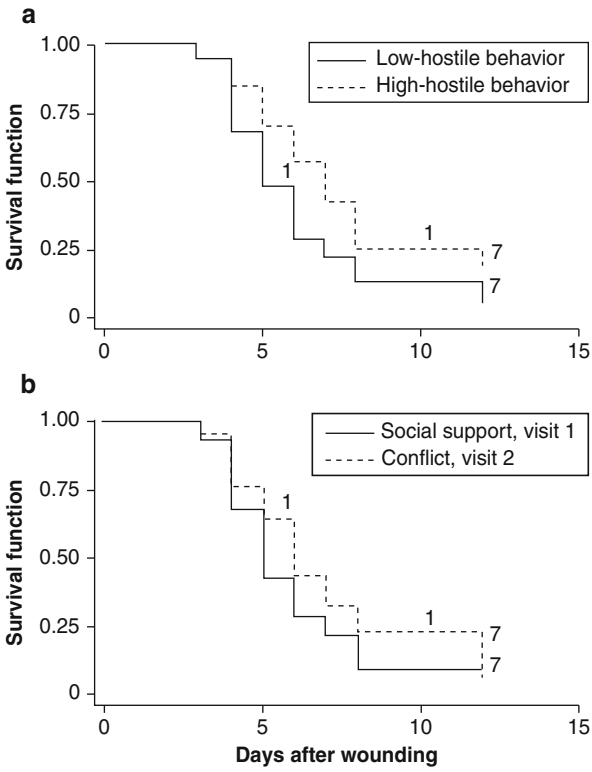

Fig. 4.1 Kaplan–Meier survival curves for time to healing of the standard wound across both visits by high- or low-hostile behavior (**a**) and for time to healing of the standard wound by visit (**b**). Couples who demonstrated consistently higher levels of hostile behaviors across both their interactions healed at 60% of the rate of low-hostile couples, and healing during the conflict visit was at 72% of the rate observed following the social support visit. Annotations on the curves show the number of censored observations at each point, indicating patients who were unhealed on their last day of observation (Reprinted from Kiecolt-Glaser et al. 2005)

skin and the ability of the skin to heal quickly following injury are important measures of immune competence. When tissue damage occurs in healthy people, healing progresses sequentially through three overlapping phases: inflammation, proliferation, and remodeling (Baum and Arpey 2005; Singer and Clark 1999). Success in later phases is highly dependent on preceding phases. A variety of stressors can delay wound healing in humans and there is evidence that this is mediated, in part, by effects of stress on the inflammatory stage of the healing process.

In one study, we found that women experiencing the chronic stress of caregiving for a spouse with dementia required 24% longer to heal a small standardized punch biopsy wound than controls (Kiecolt-Glaser et al. 1995). Similarly, dental students who received mucosal punch biopsy wounds in the hard palate healed an average of 40% more slowly during an academic examination period than during a vacation period, which was rated as less stressful by participants (Marucha et al. 1998). Stress of an even shorter duration has measurable effects on healing (Fig. 4.1). In a study of married couples, participants healed more slowly when they completed a 30-min conflictive interaction with their spouses in a laboratory setting than when they completed a supportive interaction (Kiecolt-Glaser et al. 2005). Those who demonstrated consistently high levels of hostile behavior during both interactions healed wounds at 60% of the rate of low-hostile individuals.

Each of the studies described above also provides evidence that stress disrupts the inflammatory stage of healing; in this context, a robust local inflammatory response is beneficial. Among married couples, decreased production of three key cytokines – IL-6, IL-1β, and TNF-α – was observed at the wound site following the conflictive compared to supportive interaction. Similarly, compared to controls, circulating peripheral blood leukocytes (PBLs) from caregivers expressed less IL-1β-encoded messenger RNA (mRNA) in response to lipopolysaccharide (LPS) stimulation (Kiecolt-Glaser et al. 1995). Finally, in the study with dental students, production of IL-1β mRNA by LPS-stimulated PBLs was lower in every dental student during exams compared to vacation.

In addition to studies of wound healing, effects of stress on skin function can also be assessed using tape stripping, a minimally invasive procedure. Through repeated applications of cellophane tape, commonly to the forearm, a layer of epithelial skin cells is removed from the outermost layer (stratum corneum) causing mild skin barrier disruption. Tape stripping impairs the skin's ability to regulate moisture, resulting in greater transepidermal water loss (TEWL) (Marks 2004). Skin barrier recovery can be quantified by measuring changes in TEWL over time.

Several studies have demonstrated that stress slows skin barrier recovery following tape stripping. Dental students recovered significantly more slowly when assessed during academic exams as compared to vacation with approximately 30% versus 45% recovery at 3 h following tape stripping (Garg et al. 2000). Brief laboratory stressors such as public speaking also reduce the speed of skin barrier recovery by 10–15% (Altemus et al. 2001; Robles 2007).

The magnitude to which the same stressor exerts an impact on healing may differ across individuals. Dental students reporting the greatest stress during academic exams showed the greatest decrements in skin barrier recovery following tape stripping (Garg et al. 2000). Conversely, certain traits may buffer individuals from negative effects of stress; individuals high in trait positive affect did not show decrements in skin barrier recovery upon exposure to an acute laboratory stressor, although decrements were seen among those low in trait positive affect (Robles et al. 2009).

Together, data from these studies show that objective stressors ranging from chronic (e.g., caregiving) to acute (e.g., brief laboratory task) significantly impact healing and skin barrier recovery. Further, subjective ratings of stress as well as individual characteristics (e.g., hostility, trait positive affect) can affect the magnitude of such effects. Thus, although the experience of objective stressors affects healing, coping abilities, and personality characteristics that promote resilience may buffer such effects, making some individuals less susceptible to stress-induced decrements in healing.

4.3 Stress and Infectious Agents

Central to immune function is the ability to respond quickly and adequately in response to infectious agents. As described below, it is well established that stress not only makes individuals more susceptible to illness after exposure to infectious

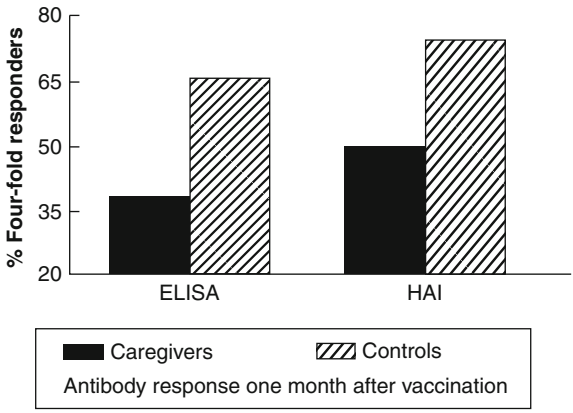

Fig. 4.2 Percentage of caregivers and controls showing a clinically significant fourfold increase in antibody 1 month after seasonal influenza virus vaccination using antibody data from two different assays (Reprinted from Kiecolt-Glaser et al. 1996). *HAI* hemagglutination-inhibition assay

agents, but can also inhibit the antibody and virus-specific T-cell response to vaccines and can induce the reactivation of latent herpes viruses.

4.3.1 Vaccination Studies

Psychological stress predicted poorer immune responses to vaccines including influenza, pneumococcal pneumonia, hepatitis B (Hep B), and meningococcal C (Glaser et al. 1998, 2000; Kiecolt-Glaser et al. 1996; Vedhara et al. 1999; Phillips et al. 2005; Burns et al. 2002, 2003; Glaser et al. 1992; Miller et al. 2004; Christian et al. 2009; Cohen et al. 2001; Pedersen et al. 2009). This can result in inadequate protection upon exposure to the infectious agent of interest (Couch and Kasel 1983). Poor antibody responses to a bacterial vaccine (pneumococcus) and poor antibody and virus-specific T-cell responses to viral vaccines (influenza and Hepatitis B) also serve as a marker of diminished immune function in general, which may have implications beyond vulnerability to a specific infectious illness (Kiecolt-Glaser et al. 1996).

Effects of caregiving stress on antibody responses to influenza virus vaccination have been demonstrated by our group and others in elderly and nonelderly caregivers (Fig. 4.2) (Vedhara et al. 1999, 2002; Glaser et al. 1998; Kiecolt-Glaser et al. 1996; Segerstrom et al. 2008). In addition, greater perceived stress and more frequent stressful life events have been associated with lower antibody responses and poorer maintenance of antibody levels following influenza vaccination among young adults (Phillips et al. 2005; Burns et al. 2003; Miller et al. 2004). A recent meta-analysis of 13 studies concluded that the effect of stress on antibody responses to influenza virus vaccination corresponded to adequate antibody responses among 41% of stressed individuals versus 59% of less stressed individuals with similar effects among older and younger adults (Pedersen et al. 2009).

Life stress has also been associated with poorer maintenance of antibody levels against a pneumococcus vaccine (Glaser et al. 2000), poorer seroconversion and maintenance of antibody against Hep B (Burns et al. 2002; Glaser et al. 1992), and slower antibody responses to meningococcal C vaccination (Phillips et al. 2005). Although detailed coverage of neuroendocrine mechanisms underlying these associations is beyond the scope of the current chapter, animal models demonstrate multiple effects of glucocorticoid hormones on cell-trafficking, and production of proinflammatory cytokines and chemokines which contribute to these effects (for review see Godbout and Glaser 2006; Padgett and Glaser 2003).

4.3.2 Infectious Illness

Stress affects susceptibility, severity, and duration of infectious illnesses including influenza and the common cold virus. A number of naturalistic studies have reported associations between stress and frequency of infectious illness (e.g., Turner-Cobb and Steptoe 1996; Graham et al. 1986). However, the strongest evidence of effects of stress on infectious illness comes from controlled laboratory studies in which participants have been exposed to infectious agents. In a key study, Cohen and colleagues demonstrated that self-reported stress predicted susceptibility to respiratory viruses in a dose–response manner. Healthy subjects were exposed to one of five respiratory viruses, quarantined, and assessed for respiratory symptoms and virus-specific antibody titers. Those reporting greater stress (a composite measure of major life events, perceptions of stress, and negative affect) showed greater likelihood of developing respiratory infections as well as clinically defined colds (Cohen et al. 1991). This effect was found across each of the five types of virus strains, and the effect remained after controlling for numerous potential confounding factors. Subsequent research from the same laboratory demonstrated that chronic life stress lasting 1 month or longer is key to such effects (Cohen et al. 1998).

Individuals who experience greater physiological reactivity when facing stressful situations may be more vulnerable to infectious illness. Among 115 healthy individuals, those who showed larger cortisol responses to a laboratory stressor experienced greater risk of developing clinically verified colds under conditions of higher stress (Cohen et al. 2002). Stress level was unrelated to risk of colds among those showing smaller cortisol responses to acute stress.

In addition to affecting risk of illness, stress can affect symptom severity in part by causing dysregulation of inflammatory responses. In a study of people experimentally exposed to an influenza virus, those reporting higher stress experienced greater symptoms of illness, greater mucus weight, as well as greater inflammatory responses to the infection, as indicated by higher IL-6 levels in nasal secretions (Cohen et al. 1999).

4.3.3 Latent Viruses

After exposure to most infectious agents, the immune system clears the body of the pathogen. However, some viruses become cell associated after primary infection,

where they remain for life. This is known as latency. These include herpes viruses, such as herpes simplex virus (HSV) I and II, varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and cytomegalovirus (CMV). One of the most prevalent latent viruses, EBV, is carried by more than 95% of the adult North American population (Wolf and Morag 1998). Reactivation of latent EBV typically causes little or no symptoms in healthy individuals (Hess 2004). For example, reactivation of HSV I or II can cause cold sores (Bystricka and Russ 2005). However, in some instances, severe symptoms can result as is the case of VZV reactivation which can cause shingles (Quinlivan and Breuer 2006). Even in the absence of clinical disease, reactivation of a latent virus provides a sensitive marker for impairment/deficiencies in cell-mediated immunity. Thus, studies of viral latency and viral-specific markers can provide clinically relevant information even among asymptomatic individuals.

As seen with other markers of immune dysfunction such as virus-specific antibody titers, caregivers show more evidence of reactivation of latent viruses including EBV and HSV-1 compared to well-matched controls (Kiecolt-Glaser et al. 1991; Glaser and Kiecolt-Glaser 1997). The stress associated with disruption of significant relationships also affects viral latency. Women who were recently divorced or separated showed poorer control of latent EBV than demographically matched married women (Kiecolt-Glaser et al. 1987). Similarly, men who were unsatisfied in their marriage had higher levels of EBV antibody titers than their happily married counterparts (Kiecolt-Glaser et al. 1988).

Stressors of a more transient nature also affect viral latency. In prospective studies, medical students exhibited higher EBV, HSV-1, and CMV antibody titers on the day of an academic examination, as compared to several weeks before or after the exam (Glaser et al. 1985, 1999; Sarid et al. 2001).

In addition to effects of objective stressors, psychological factors have been associated with immune control of latent virus. Higher levels of EBV-specific antibodies are reported among those with a greater tendency to repress their emotions (Esterling et al. 1990), higher levels of anxiety (Esterling et al. 1993), and greater loneliness (Glaser et al. 1985). Similarly, individuals with syndromal or subsyndromal symptoms of depression have shown higher levels of HSV-1 antibody and poorer VZV-specific T-cell immunity than those without depressive symptoms (Delisi et al. 1986; Robertson et al. 1993; Irwin et al. 1998). Conversely, among older women undergoing the stressor of housing relocation, higher self-reported vigor was associated with better immune control of latent EBV (Lutgendorf et al. 2001). Thus, in addition to objective stressors, certain psychological characteristics (e.g., mood, ways of coping) may affect immune control of latent viruses. Physiological mechanisms by which stress may impair control of latent viruses include impairment of cytotoxic and proliferative T-cells responses (Glaser et al. 1987, 1993).

4.4 Interventions

Given the clear negative impact of stress on immune parameters, interventions addressing stress from a psychosocial, physical, nutritional/dietary, and pharmacological perspective are receiving attention (see also chapters in Part V of this volume).

Stress management groups which involve elements of social support, emotional disclosure, and a focus on problem solving have improved antibody responses to influenza vaccination among caregivers (Vedhara et al. 2003) and immune control of latent viruses among HIV-infected (Lutgendorf et al. 1997; Esterling et al. 1992; Carrico et al. 2005; Cruess et al. 2000). In addition, stress reduction through relaxation training improved immune control of latent HSV-1 among older adults (Kiecolt-Glaser et al. 1985). Among healthy adults, an 8-week meditation intervention prior to influenza vaccination resulted in better antibody responses as compared to a waiting-list control group (Davidson et al. 2003). A 16-week Tai Chi intervention, which involves physical activity as well as meditation, improved mental health and antibody responses to VZV vaccine among older adults (Irwin et al. 2007). We found that regular practice of yoga, which also involves elements of meditation and physical activity, is associated with reduced proinflammatory cytokine activity; compared to women with a regular yoga practice, women with limited yoga experience had 41% higher IL-6 levels, 4.75 times greater likelihood of detectable CRP levels, and greater stress-induced in vitro cytokine production (Kiecolt-Glaser et al. 2010). Similarly, a study of mindfulness-based stress reduction for breast cancer patients, which included elements of meditation and gentle yoga, demonstrated improvements in mood, reductions in perceived stress, and beneficial immunological changes including decreased production of inflammatory cytokines and increased production of anti-inflammatory cytokines by stimulated T-cells (Carlson et al. 2003).

Other research has examined the ability of physical/aerobic exercise to foster immune responses: Older adults who completed a 3-month aerobic exercise intervention healed standard punch biopsy wounds 25% more quickly than did their less active counterparts (Emery et al. 2005).

Another important avenue for counteracting or preventing the negative effects of psychological stress is diet. Among others, omega-3 (*n*-3) polyunsaturated fatty acids (PUFAs) are an important dietary factor for mood and immune function. Due to their inhibitory effects on proinflammatory cytokine production (Logan 2003), higher *n*-3 PUFA levels are related to lower levels of circulating proinflammatory cytokines (Ferrucci et al. 2006; Kiecolt-Glaser et al. 2007). Via effects of inflammatory processes, *n*-3 supplementation may help to prevent stress-induced depressive symptoms (for review, see Parker et al. 2006). Nutritional and further pharmacological interventions warrant further investigation to reduce and modulate stress-dependent negative effects on distinct immune functions (see also Chaps. 29 and 30).

In sum, a variety of interventions show promise for counteracting the negative effects of psychological stress. The particular intervention (i.e., stress management, physical activity, meditation) which is most beneficial likely depends on the outcome of interest, type of stressors experienced, as well as personality characteristics and preexisting behaviors of the person in question. An emphasis of future research should be on targeted, individualized risk assessments and interventions.

4.5 From Daily Life to Space Travel

Clearly, daily life stressors ranging in magnitude and duration affect clinically meaningful health outcomes including inflammatory processes, wound healing, and responses to infectious agents and other immune challenges (e.g., vaccination). Individuals vary in their ability to cope with stress, and differences in perceptions of stress, mood (e.g., depressive symptoms), and personality (e.g., hostility) can modify the magnitude to which stressors exert a negative influence on immune function. Research provides support for several promising avenues for interventions to prevent stress-induced immune dysregulation. However, more information is needed to provide individualized intervention strategies.

Space travel presents an exceptional and intense combination of physical and psychological challenges. It provides a unique opportunity to examine the susceptibility of the human body to stress and to explore interventions promoting psychological and physiological resilience. Thus, studies of daily life stress inform our understanding of potential impacts of the stressors of space travel. In turn, studies of stressors encountered in space travel can also benefit our understanding of stress in our daily lives.

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Part III

Stress and Immune Allostasis in Space, from Brain to Immune Responses

5 Neurobiological Mechanisms of Stress and Glucocorticoid Effects on Learning and Memory: Implications for Stress Disorders on Earth and in Space

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5.1 Introduction

Space flight conditions affect human health due to complex environmental challenges ("stressors"). As discussed elsewhere in this book, adequate function of human organisms in space is challenged by both biological (e.g., sleep deprivation, pain) and physical stress factors (e.g., microgravity, variable oxygenation status, radiation). It has been long recognized that stress leads to an activation of the sympathetic nervous system and hypothalamus-pituitary adrenal (HPA) axis, culminating in the release of catecholamines and glucocorticoids (i.e., cortisol) from the adrenal medulla and cortex, respectively (De Boer et al. 1990; McCarty and Gold 1981; Roozendaal et al. 1996a). These hormones are known to influence the organism's ability to cope with stress, influencing target systems in the periphery. However, these hormones also induce a myriad of effects on the brain. Accordingly, high stress exposure is known to affect emotional regulation, cognitive function, and mood. Glucocorticoid hormones play a crucial role in the regulation of aversive memory and mood, thereby influencing the development and symptoms of anxiety disorders (Aerni et al. 2004; Schelling et al. 2004b; Soravia et al. 2006). A few studies investigated the occurrence of psychological

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problems during space flight conditions. Noted problems included anxiety, boredom, crew interactions, problems associated with isolation and confinement, and others (Cooper 1996). Likewise, simulation studies of manned space flight on earth reported the incidence of anxiety, depression, psychosis, psychosomatic symptoms, emotional reactions related to mission stage, asthenia, and post-flight personality and marital problems (Kanas 1997). However, despite extensive evidence that learning and memory processes play a crucial role in the development of several of these psychological problems, an assessment of learning and memory functions is not typically performed. Nevertheless, it is obvious that an altered emotional, cognitive, or mood status during highly demanding tasks, especially during prolonged periods, could add to the subjective feeling of perceived stress and further impact human health via neuroendocrine and nerval-immune modulatory effects as well as might induce long-lasting impairment of psychological well-being. Therefore, we encourage the implementation of cognitive and mood monitoring aboard future space missions.

In this chapter, we will describe the current status of our understanding of the neurobiological mechanisms that are involved in regulating stress and glucocorticoid effects on memory processes and why these stress hormones might specifically modulate memory of emotionally arousing experiences. Furthermore, because emotional memory plays a crucial role in the pathogenesis and symptomatology of anxiety disorders, such as posttraumatic stress disorder (PTSD), which is linked to a variety of immune changes as well, we will discuss to what extent the basic findings on glucocorticoid effects on emotional memory might have clinical implications on earth and in space.

5.2 Glucocorticoid Effects on Learning and Memory

Early reports on both enhancing and impairing properties of glucocorticoids on memory (Arbel et al. 1994; Beckwith et al. 1986; Bohus and Lissak 1968; Flood et al. 1978; Luine et al. 1993) have indicated that these hormones have complex effects on cognitive functions. More recent studies investigating glucocorticoid effects on distinct memory phases and studies discerning acute from chronic effects helped to disentangle the multifaceted actions of these stress hormones. For example, acute elevations of glucocorticoids are known not only to enhance the consolidation of memory of new information, but to impair the retrieval of previously stored information (de Quervain et al. 1998a; Roozendaal and McGaugh 1996). Conditions with chronically elevated glucocorticoid levels are usually associated with impaired cognitive performance and these deficits are thought to result from a cumulative and long-lasting burden on hippocampal function and morphology (McEwen 2001; Sapolsky 2000). Recently, however, it became clear that memory deficits observed under such chronic conditions can also result, at least in part, from acute and reversible glucocorticoid actions on memory retrieval processes (Coluccia et al. 2008). Some of the effects of glucocorticoids on cognitive performance might be mediated via influences on immune regulators. It is well established that cytokines as well as other immune factors affect learning and memory functions (Cibelli et al. 2010;

Derecki et al. 2010). These findings indicate that an integrated prospective is necessary to fully understand the impact of stress and glucocorticoids on cognition and health. As long-duration space flight is often associated with both high stress and an impacted immune function, such interactions might be especially relevant in these conditions.

5.2.1 Glucocorticoid Effects on Memory Consolidation

Memory consolidation is the process by which a fragile short-term memory trace is transferred into stable long-term memory. However, not all information is equally well transferred into long-term storage. In fact, it is well recognized that especially emotionally arousing experiences are well remembered, even after decades (McGaugh 2003). There is extensive evidence from studies in animals and humans that glucocorticoids, along with other components of the stress response, are critically involved in regulating memory consolidation of emotionally arousing experiences (McGaugh and Roozendaal 2002). Acute systemic administration of glucocorticoids enhances long-term memory consolidation when given either before or immediately after a training experience (Abercrombie et al. 2003; Buchanan and Lovallo 2001; Cordero et al. 2002; Roozendaal and McGaugh 1996). In contrast, a blockade of glucocorticoid production with the synthesis inhibitor metyrapone impairs memory consolidation (Maheu et al. 2004; Roozendaal et al. 1996a) and prevents stress – and epinephrine-induced memory enhancement (Liu et al. 1999; Roozendaal et al. 1996b). Such glucocorticoid effects on memory consolidation follow an inverted U-shape dose–response relationship: Moderate doses enhance memory, whereas higher doses are typically less effective or may even impair memory consolidation (Roozendaal et al. 1999b).

5.2.1.1 Role of Emotional Arousal in Enabling Glucocorticoid Effects on Memory Consolidation

Recent findings from animal and human studies suggest that glucocorticoids modulate memory consolidation of emotionally arousing experiences but do not affect memory consolidation of neutral information. We investigated the importance of emotional arousal in mediating glucocorticoid effects on memory consolidation in rats trained on an object recognition task (Okuda et al. 2004). Although no rewarding or aversive stimulation is used during object recognition training, such training induces modest novelty-induced stress or arousal (De Boer et al. 1990). However, extensive habituation of rats to the training apparatus prior to the training reduces the arousal level induced by object recognition training. We found that corticosterone administered systemically immediately after training enhanced 24-h retention performance of rats that were not previously habituated to the experimental context (i.e., emotionally aroused rats). In contrast, posttraining corticosterone did not affect 24-h retention of rats that had received extensive prior habituation to the experimental context and, thus, had decreased novelty-induced emotional arousal during training (Okuda et al. 2004) (Fig. 5.1). A link between the level of emotional arousal at encoding and the

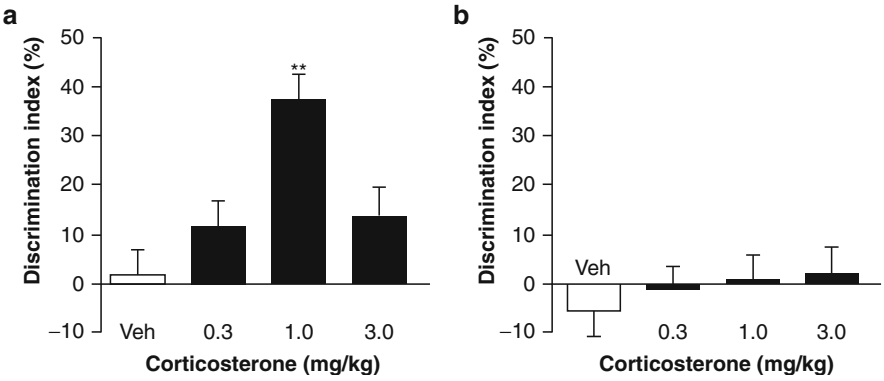

Fig. 5.1 The enhancing effect of posttraining administration of corticosterone on 24-h object recognition performance depends on emotional arousal. Rats received a single injection of corticosterone or vehicle immediately after the 3-min training trial. Corticosterone administered in a dose of 1.0 mg/kg significantly enhanced 24-h object recognition memory of aroused rats in the WITHOUT-habituation condition (**a**) but failed to affect memory of non-aroused rats in the WITH-habituation condition (**b**). ** $P < 0.0001$ compared with the corresponding vehicle control group. Data are presented as mean ± SEM (Reprinted from Okuda et al. 2004)

efficacy of adrenocortical stress hormones in influencing memory consolidation has also been demonstrated in humans. Cortisol administered shortly before or after learning selectively enhances long-term memory of emotionally arousing, but not of emotionally neutral, items (Buchanan and Lovallo 2001; Kuhlmann and Wolf 2006a). Moreover, a cold pressor stress in humans (i.e., placing the arm in ice water for up to 3 min), a procedure that significantly elevates endogenous cortisol levels, enhanced memory of emotionally arousing slides, but did not affect memory of relatively neutral slides (Cahill et al. 2003). Consistent with these findings, it has been reported that levels of endogenous cortisol at the time of learning correlated with enhanced memory consolidation only in individuals who were emotionally aroused (Abercrombie et al. 2006). Thus, these findings from animal and human studies indicate that training-associated endogenous emotional arousal is essential for enabling glucocorticoid effects on memory consolidation.

5.2.1.2 Role of Arousal-Induced Noradrenergic Activation in the Amygdala

Why do stress hormones selectively enhance memory consolidation of emotionally arousing experiences? Our findings suggest that interactions between stress hormones and amygdala activity may be key in determining this selectivity (see also Chap. 3). It is well established that emotional experiences that induce the release of adrenal stress hormones also activate the amygdala (Pelletier et al. 2005). Evidence from a large number of studies in both animals and humans has indicated that the amygdala plays a critical role in the induction of the stress response via influences on hypothalamic and brainstem control centers as well as in the processing of emotionally arousing information, including emotional influences on attention, perception,

and learning and memory (LeDoux 2003; Phelps and LeDoux 2005). Extensive evidence from our as well as other laboratories indicates that the enhancing effects of stress hormone administration on the consolidation of memory of emotionally arousing experiences involve the amygdala. Lesions of the basolateral complex of the amygdala (BLA) block the memory-modulatory effects induced by posttraining systemic injections of glucocorticoids (Roozendaal and McGaugh 1996). Moreover, and in support of this view, posttraining infusions of a specific glucocorticoid receptor agonist administered into the BLA enhance memory consolidation, whereas intra-BLA infusions of a glucocorticoid receptor antagonist impair memory consolidation (Roozendaal and McGaugh 1997b). Further findings indicate that glucocorticoids require training-associated noradrenergic activation within the BLA to influence memory of emotionally arousing training. In vivo microdialysis, a technique used to measure ongoing changes in neurotransmitter release in the brain, indicated that aversive stimulation of electric foot shock induces the release of norepinephrine in the amygdala (McIntyre et al. 2002). Blockade of the norepinephrine signaling pathway in the BLA with a β-adrenoceptor antagonist prevented the memory-enhancing effect of systemically administered glucocorticoids (Quirarte et al. 1997; Roozendaal et al. 2002, 2006a, b). These findings strongly suggest that because glucocorticoid effects on memory consolidation require noradrenergic activation within the BLA, they only modulate memory under emotionally arousing conditions that induce the release of norepinephrine (Roozendaal et al. 2006b). Human studies have also provided evidence that stress hormone effects on memory enhancement for emotionally arousing experiences require amygdala and noradrenergic activity (Adolphs et al. 1997; Cahill et al. 1995, 1996; Canli et al. 2000; Hamann et al. 1999).

5.2.1.3 Interaction of the Amygdala with Other Brain Regions

Although evidence suggests that the BLA might be a critical site involved in the encoding, storage, and expression of fear-related memories (LeDoux 2003), an involvement of the BLA in modulating long-term memory formation has been obtained in experiments using many different kinds of training tasks. As these different training experiences are known to engage different brain systems (Izquierdo et al. 1997; McGaugh 2002; Packard et al. 1994; Packard and Knowlton 2002), the BLA-induced modulation no doubt involves influences on processing occurring in these brain regions (McGaugh and Roozendaal 2002). For example, glucocorticoids administered into the hippocampus influence memory of water-maze spatial training (Roozendaal and McGaugh 1997a), which is consistent with a role of the hippocampus in spatial/contextual learning and memory (Maren and Fanselow 1997; Morris et al. 1982; Sacchetti et al. 1999). Glucocorticoids infused into the hippocampus also enhance memory of inhibitory avoidance training (Roozendaal and McGaugh 1997a; Roozendaal et al. 1999a). Importantly, lesions of the BLA or the administration of a β-adrenoceptor antagonist into the BLA block the enhancing effect of posttraining intra-hippocampal infusions of a glucocorticoid receptor agonist on inhibitory avoidance memory (Roozendaal and McGaugh 1997a; Roozendaal et al. 1999a). Thus, these findings indicate that an intact and functional BLA is

required for enabling memory modulation of spatial/contextual information induced by manipulation of glucocorticoid receptor activity in the hippocampus. In other experiments, we found that corticosterone enhances memory consolidation of conditioned taste aversion when infused posttraining into the insular cortex or the BLA, but is ineffective when administered into the hippocampus, a region that does not play a significant role in taste learning (Miranda et al. 2008). Taken together, these findings indicate that glucocorticoids act in different brain regions to enhance the consolidation of different aspects of information acquired during training. Although the BLA plays a critical role in regulating stress hormone effects on memory consolidation, extensive evidence indicates that it is not a permanent storage site of such memory traces (McGaugh 2004), but rather interacts with these other brain regions in regulating memory consolidation of different kinds of information (McGaugh 2002).

5.2.2 Glucocorticoid Effects on Memory Retrieval

Many studies indicate that stress and glucocorticoids not only modulate the strength of newly formed memories, but also influence the remembrance of previously acquired information. In contrast to the enhancing effects of glucocorticoids on memory consolidation, these hormones typically impair memory retrieval. In the first study investigating the effects of stress and glucocorticoids on retrieval processes (de Quervain et al. 1998b), it was reported that 30 min after exposure to footshock stress, rats had impaired retrieval of spatial memory of a water-maze task they had acquired 24 h earlier. The water-maze task is a commonly used learning task in which rats make use of spatial cues in their environment to learn to navigate to an invisible (i.e., submerged) escape platform in a tank filled with water. Interestingly, memory performance was not impaired when rats were tested either 2 min or 4 h after the stress exposure. These time-dependent effects on retrieval processes corresponded to the circulating glucocorticoid levels at the time of testing, which suggested that the retrieval impairment was directly related to increased adrenocortical function. In a next step, these findings were translated to healthy humans and found that a single administration of cortisone impaired the recall of words learned 24 h earlier (de Quervain et al. 2000). Several further studies from different laboratories have indicated that glucocorticoids impair memory retrieval of spatial or contextual memory in rats and declarative (mostly episodic) memory in humans (Buss et al. 2004; Coluccia et al. 2008; de Quervain et al. 2003; Het et al. 2005; Kuhlmann et al. 2005a; Rashidy-Pour et al. 2004; Roozendaal et al. 2003, 2004b; Sajadi et al. 2007; Wolf 2008; Wolf et al. 2001). Moreover, increased cortisol levels after psychological stress exposure have also been shown to impair declarative memory retrieval (Buchanan et al. 2006; Domes et al. 2004; Kuhlmann et al. 2005b).

An important question is whether glucocorticoid effects on memory retrieval occur only under acutely elevated glucocorticoid levels or also under chronic conditions. There is substantial evidence that sustained endogenous hypercortisolemia, such as found in depression, Cushing's disease, or human aging, is often associated

with declarative memory impairment (Lupien et al. 1998; Rubinow et al. 1984; Seeman et al. 1997; Starkman et al. 1992). Moreover, prolonged glucocorticoid therapy, which is widely used in clinical practice, is known to induce cognitive deficits (Bermond et al. 2005; Brown et al. 2004; Brunner et al. 2005; Keenan et al. 1996; Wolkowitz et al. 1997). Although considerable evidence suggests that long-term glucocorticoid exposure may cause cognitive impairment via cumulative and long-lasting influences on hippocampus function and morphology (Brown et al. 2004; Joëls 2001; McEwen 2001; Sapolsky 2000; Starkman et al. 1992), it is possible that also acute hormonal influences on retrieval processes contribute to the memory deficits found with chronic glucocorticoid exposure. To investigate this question, memory functions and hippocampal volume were examined in patients with rheumatoid arthritis who were treated either chronically (i.e., for a mean duration of 5 years) with low-to-moderate doses of prednisone or without glucocorticoids. In both groups, delayed recall of words learned 24 h earlier was assessed under conditions of either elevated or basal glucocorticoid levels in a double-blind, placebo-controlled crossover design. The findings did not indicate harmful effects of a history of chronic prednisone treatment on memory performance or hippocampal volume (i.e., no differences were found between both groups when retention was tested under placebo treatment). However, acute prednisone administration 1 h before retention testing to either the steroid or nonsteroid group impaired verbal recall (Coluccia et al. 2008). Thus, these findings indicate that acute and reversible effects of glucocorticoids on retrieval processes contribute to the memory deficits found in conditions of chronically elevated glucocorticoid levels.

5.2.2.1 Role of Amygdala-Hippocampal Interaction in Enabling Glucocorticoid Effects on Memory Retrieval

Extensive evidence from studies in amnesic patients, human imaging studies, and lesion studies in animals indicates that the medial temporal lobe (hippocampus and parahippocampal gyrus) is crucially involved in the retrieval of spatial and contextual memory in animals and declarative memory in humans (Cabeza and Nyberg 2000; Moser and Moser 1998; Squire 1992). Systemic administration of glucocorticoids to rats shortly before retention testing induces retrieval impairments for contextual and spatial memory (de Quervain et al. 1998a; Roozendaal et al. 2004a). Furthermore, local infusions of a glucocorticoid receptor agonist into the hippocampus of rats induce memory retrieval impairment on a water-maze spatial task comparable to that seen after systemic administration (Roozendaal et al. 2003). In parallel, a positron-emission tomography (PET) imaging study in healthy humans showed that acutely administered cortisone reduces blood flow in the medial temporal lobe during memory recall of words, an effect that correlated with the degree of memory retrieval impairment (de Quervain et al. 2003). Moreover, a recent functional magnetic resonance imaging (fMRI) study found that glucocorticoids decrease hippocampal and prefrontal activation during declarative memory retrieval (Oei et al. 2007).

Other studies indicated that glucocorticoid effects on memory retrieval also depend on emotional arousal and BLA activity. Specifically, it has been shown in recent studies

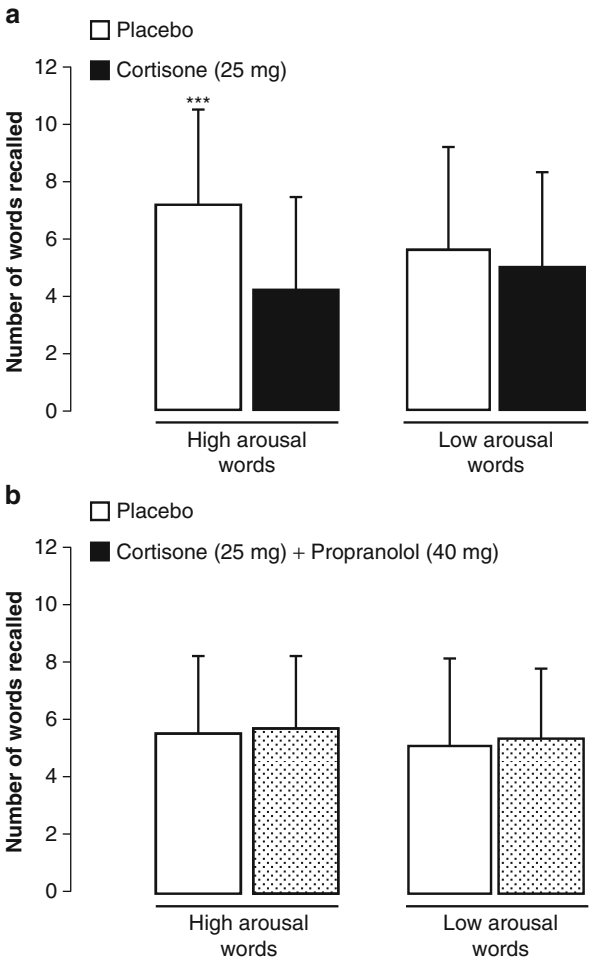

Fig. 5.2 Glucocorticoid effects on memory retrieval depend on emotional arousal. (**a**) Cortisone administered 1 h before recall testing impaired retrieval of high-arousal words, but not of low-arousal words. (**b**) Concurrent administration of the β-blocker propranolol prevented the impairing effect of cortisone on high-arousal words. Data are presented as mean ± SEM. *** $P = 0.0001$ compared with the corresponding cortisone condition (Adapted from de Quervain et al. 2007a)

in humans that emotionally arousing information is especially sensitive to the retrieval-impairing effects of glucocorticoids (Fig. 5.2) (de Quervain et al. 2007a; Kuhlmann et al. 2005a, b; Smeets et al. 2008), and that emotional arousal during the test situation enables glucocorticoid effects on memory retrieval (Kuhlmann and Wolf 2006b). Studies of rats investigating the neural mechanisms underlying this selectivity have indicated that glucocorticoid effects on memory retrieval depend critically on arousal-induced noradrenergic activity within the brain (Roozendaal et al. 2004a, b). Infusion of a β-adrenoceptor antagonist into the BLA blocks the impairing effect of a glucocorticoid receptor agonist infused into the hippocampus on memory retrieval of spatial information (Roozendaal et al. 2003, 2004b). In line with this idea, the β-adrenoceptor antagonist propranolol blocked the impairing effect of cortisone on the retrieval of emotionally arousing verbal material in healthy humans (Fig. 5.2) (de Quervain et al. 2007a). These findings may have important clinical implications as β-adrenoceptor

antagonists might prove useful for the prevention of glucocorticoid-induced memory deficits in acute stressful situations (Kuhlmann et al. 2005b), as well as for the treatment of memory deficits in conditions associated with chronically elevated glucocorticoid levels, such as depression or medical conditions requiring glucocorticoid treatment (Coluccia et al. 2008; Roozendaal and de Quervain 2005).

5.2.3 Glucocorticoid Effects on Working Memory

Working memory is a dynamic process whereby information is updated continuously, providing a temporary storage of information (Baddeley 1992; Jones 2002). Evidence from lesion, pharmacological, imaging, and clinical studies indicates that working memory depends on the integrity of the prefrontal cortex (Brito et al. 1982; Fuster 1991; Owen et al. 2005). Stress exposure is known to impair performance of rats on a delayed alternation task, a task commonly used to assess working memory in rodents (Arnsten and Goldman-Rakic 1998). Although basal levels of endogenous glucocorticoids are required to maintain prefrontal cortical function (Mizoguchi et al. 2004), systemic administration of stress doses of glucocorticoids impairs delayed alternation performance in rats (Roozendaal et al. 2004c). Stress or stress-level cortisol treatment is also known to impair working memory performance in human subjects during demanding tasks that require a high level of arousal (Baddeley 1992; Lupien et al. 1999; Schoofs et al. 2008; Wolf et al. 2001; Young et al. 1999). Importantly, like glucocorticoid effects on memory consolidation and retrieval, these hormones interact with noradrenergic mechanisms in inducing working memory impairment (Roozendaal et al. 2004c). Animal studies have shown that glucocorticoid effects on working memory also depend on functional interactions between the BLA and the medial prefrontal cortex. Disruption of BLA activity blocks the effect on working memory of a glucocorticoid receptor agonist administered into the medial prefrontal cortex (Roozendaal et al. 2004c). This evidence provides strong support for the hypothesis that BLA activity modulates stress- and emotional arousal effects on working memory in other brain regions.

5.3 Modulatory Effects of Glucocorticoids on Emotional Memory: Implications for Anxiety Disorders

From the findings reviewed above, we have learned that glucocorticoids enhance memory consolidation but impair memory retrieval and working memory in emotionally arousing situations (Fig. 5.3). Enhanced memory for emotional events is a well-recognized phenomenon, which helps us to remember important information. Although glucocorticoid-induced temporary impairments of memory retrieval and working memory may directly negatively influence task performance during highly stressful conditions, these effects should not a priori be regarded biologically as

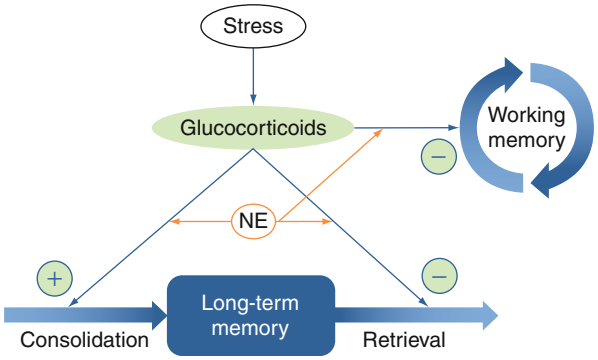

Fig. 5.3 Effects of stress and glucocorticoids on memory functions. Whereas glucocorticoids enhance memory consolidation, they impair memory retrieval and working memory. All of these hormone effects depend on emotional arousal-induced activation of noradrenergic transmission. *NE* norepinephrine (Reprinted from (de Quervain et al. 2009))

maladaptive. In fact, these effects may actually aid to an accurate storage of emotionally arousing information by blocking, for example, retroactive interference (Roozendaal 2002). However, whereas the enhanced memory for emotionally arousing events in most cases has a clear adaptive value, in certain circumstances, extremely aversive experiences can also lead to highly emotional, traumatic, or fearful memories, which contribute to the development and symptoms of anxiety disorders. Therefore, understanding the basic modulatory actions of glucocorticoids on different aspects of cognition may have important implications for understanding and, possibly, treating stress-related (anxiety) disorders. Although we focus in this chapter on the implications of memory-modulatory glucocorticoid effects for anxiety disorders, earth-bound research on the role of glucocorticoids is likely to have important implications for other psychiatric disorders, such as depression or schizophrenia, as well (Belanoff et al. 2001; Newcomer et al. 1998; Rubinow et al. 1984; Starkman et al. 1981).

5.3.1 The Role of Aversive Memories in Anxiety Disorders

Several lines of evidence indicate that after an aversive experience the formation of an aversive memory trace is an important pathogenic mechanism for the development of anxiety disorders, such as PTSD or phobias (LeDoux 2003; Mineka and Oehlberg 2008; Phelps and LeDoux 2005; Pitman 1989; Yehuda and LeDoux 2007). Neuroimaging studies have shown that while the prefrontal cortex seems to be hyporesponsive, amygdala activity in response to viewing aversive information is exaggerated in patients with PTSD as compared to healthy controls and, importantly, correlates positively with later recall of the aversive information, and with PTSD symptom severity (Armony et al. 2005; Dickie et al. 2008; Francati et al. 2007; Heim and Nemeroff 2009; Rauch et al. 2000; Shin et al. 2005, 2006). These

findings are in line with the well-known role of the amygdala in the formation of emotional memory, as reviewed above. Furthermore, some evidence indicates that the administration of a β-adrenoceptor blocker, which is known to impair the consolidation of memory of emotionally arousing experiences, might be preventive with regard to the development of subsequent PTSD (Pitman et al. 2002). These findings underscore the important pathogenic role of aversive memory formation in the development of PTSD. However, the formation of a strong aversive memory trace is, of course, not sufficient to develop an anxiety disorder. In fact, building strong memories of an aversive event is a primarily adaptive mechanism and even intrusive thoughts (intrusive memory retrieval) and related symptoms are normal reactions in the first period after an aversive experience. In individuals who do not develop an anxiety disorder, which fortunately is mostly the case, intrusive memory retrieval declines over time, although the aversive memory can still be recalled voluntarily even after a long time. In contrast, in individuals who do develop a chronic anxiety disorder, the aversive memory trace remains easily reactivatable by an aversive cue (e.g., trauma cue), or even spontaneously, leading to uncontrollable aversive memory retrieval and related clinical symptoms (re-experiencing in PTSD).

PTSD is a chronic response to a traumatic event and characterized by the following features: re-experiencing of the traumatic event, avoidance of stimuli associated with the trauma, and hyperarousal. Re-experiencing symptoms include intrusive daytime recollections, traumatic nightmares, and flashbacks in which components of the event are relived (American Psychiatric Association 1994; Yehuda 2002b). These re-experiencing symptoms result from excessive retrieval of traumatic memories which often retain their vividness and power to evoke distress for decades or even a lifetime. Importantly, traumatic re-experiencing phenomena are again consolidated (re-consolidated) into memory, which cements the traumatic memory trace (see the discussion of the concept of intrusions in the PTSD research literature (e.g. Brewin 2001; Michael et al. 2005a, b). Persistent retrieval, re-experiencing, and reconsolidation of traumatic memories are stages of a process that keeps these memories vivid and thereby the disorder alive (see Sect. 5.3.3).

5.3.2 Glucocorticoids and PTSD

5.3.2.1 Preventive Effects of Glucocorticoids with Regard to the Development of PTSD

From the basic studies discussed above, we have learned that acutely elevated glucocorticoids enhance the consolidation of emotional memories. Based on these findings, it can be assumed that elevated glucocorticoid levels at the time of an aversive experience may contribute to the formation of traumatic and fearful memories. This idea is supported by a recent study on traumatic memories in critically ill patients. These patients often report traumatic memories from intensive care treatment and have a relatively high incidence of chronic stress symptoms and PTSD during follow-up (Schelling et al. 2004b). It was found that the number of traumatic memories from the intensive care unit correlated positively with the

amount of cortisol acutely administered to patients undergoing cardiac surgery (Schelling et al. 2003). Theoretically, it might therefore be useful to therapeutically block glucocorticoid signaling immediately after a traumatic incident, as has been proposed for adrenergic signaling (Pitman and Delahanty 2005). However, to block initial consolidation of aversive memories, the anti-glucocorticoid treatment should be given shortly after the aversive event. This is usually not possible and therefore this approach seems difficult. Moreover, there is evidence suggesting that reduced cortisol excretion in response to a traumatic event is actually associated with an increased risk of developing subsequent PTSD (Delahanty et al. 2000; McFarlane et al. 1997; Yehuda et al. 1998). These findings suggest that elevated glucocorticoid levels after an aversive event might be preventive with regard to the development of PTSD. This idea is strongly supported by studies showing that prolonged (several days) administration of stress doses of cortisol during intensive care treatment reduces the risk for later PTSD (Schelling et al. 2001, 2004a, 2006; Weis et al. 2006). But how do such findings of preventive effects fit with the idea that glucocorticoids enhance the formation of traumatic memories? After initial consolidation of traumatic experiences, which is likely to be enhanced by glucocorticoids, cortisol levels later on may play a crucial role in controlling the amount of retrieved traumatic memories. Specifically, by the known reducing effects of glucocorticoids on memory retrieval, high levels of these hormones may partly interrupt the vicious cycle of retrieving, re-experiencing, and reconsolidating aversive memories, thereby preventing a further cementation of the aversive memory trace. Studies showing that the preventive effects of glucocorticoid administration are also observed when the treatment started already at the time of the traumatic event (Schelling et al. 2004a; Weis et al. 2006) indicate that such an inhibitory effect of glucocorticoids on memory retrieval prevails the potentially enhancing effect on initial consolidation. Taken together, these findings suggest that elevated glucocorticoid levels (endogenously or pharmacologically) act preventively with regard to the development of PTSD. Some evidence suggests that interactions between altered glucocorticoid signaling and inflammatory responses might play a crucial role in the development of and vulnerability to PTSD (Gill et al. 2009).

5.3.2.2 Glucocorticoids Reduce the Retrieval of Traumatic Memories in Chronic PTSD

In addition to individuals at risk for PTSD, also patients with an established PTSD can show low endogenous cortisol levels (Bierer et al. 2006; Mason et al. 1986; Yehuda 2002a; Yehuda and Bierer 2008; Yehuda et al. 1995, 2007); but also see Pitman and Orr (1990); Young and Breslau (2004). A recent meta-analysis has shown that low cortisol levels depend on several factors, including gender and trauma type (Meewisse et al. 2007). Low cortisol levels may contribute to a hyper-retrieval of aversive memories. Based on the finding that glucocorticoids impair the retrieval of emotional information, we hypothesized that patients with chronic PTSD might benefit from glucocorticoid treatment. In an initial study, we tested this hypothesis in a small number of patients with chronic PTSD (Aerni et al. 2004). During a 3-month observation period, low-dose cortisol (10 mg per day) was administered orally for one month using a double-blind,

placebo-controlled, crossover design. The administration of this low dose of cortisol for one month does not cause major side effects and does not suppress endogenous cortisol production (Cleare et al. 1999). To assess possible treatment effects on the retrieval of traumatic memories, the patients rated daily the intensity and frequency of the feeling of reliving the traumatic event and the physiological distress felt in response to traumatic memories and nightmares (self-administered rating scales from the Clinician Administered PTSD Scale questions). The results of this study indicated that low-dose cortisol treatment had beneficial effects with regard to re-experiencing symptoms and nightmares and there was evidence for cortisol effects that outlasted the treatment period (Aerni et al. 2004).

5.3.3 Possible Mode of Action of Glucocorticoids in the Reduction of Traumatic Memory

The results of our clinical study suggest that glucocorticoid administration has acute effects on clinical symptoms by reducing the retrieval of traumatic memory (Aerni et al. 2004). Additionally, there was evidence that symptoms were reduced even after cessation of the treatment period. What might be the underlying mechanism? In PTSD, excessive retrieval of traumatic memory, which may be spontaneous or triggered by a trauma cue, leads to re-experiencing of the traumatic event (Michael and Ehlers 2007). (Re)consolidation of such aversive experiences further cements the aversive memory trace and thereby contributes to the persistence of the disorder (Fig. 5.4a). By inhibiting memory retrieval, cortisol may partly interrupt the vicious cycle of spontaneous retrieving, re-experiencing, and reconsolidating traumatic memories in PTSD and, thereby, promote forgetting, a spontaneous process that occurs when memory is not reactivated (Fig. 5.4b). Furthermore, cortisol may facilitate the extinction of aversive memories, as evidenced by animal studies showing that glucocorticoid signaling promotes memory extinction processes (Barrett and Gonzalez-Lima 2004; Bohus and Lissak 1968; Yang et al. 2006). Glucocorticoids may facilitate extinction in two ways: (1), because of the cortisol-induced reduction of memory retrieval, an aversive cue is no longer followed by the usual aversive memory retrieval and related clinical symptoms but, instead, becomes associated with a non-aversive experience which is stored as extinction memory; (2), because elevated glucocorticoid levels are known to enhance the long-term consolidation of memories (Buchanan and Lovallo 2001; Flood et al. 1978; Kovacs et al. 1977; Kuhlmann and Wolf 2006a; Roozendaal 2000), it is possible that glucocorticoids facilitate the storage of corrective experiences. This is supported by recent animal studies showing that post-retrieval administration of glucocorticoids is able to enhance the consolidation of extinction memory (Abrari et al. 2008; Cai et al. 2006). Theoretically, such post-retrieval (or post-reactivation) glucocorticoid effects may also be interpreted as an inhibition of reconsolidation (Tronson and Taylor 2007; Wang et al. 2008). However, findings in animals suggest that reconsolidation of aversive memory is disrupted by blocking rather than by activating glucocorticoid signaling (Tronel and Alberini 2007). Furthermore, in favor of the memory extinction hypothesis, it has been shown that

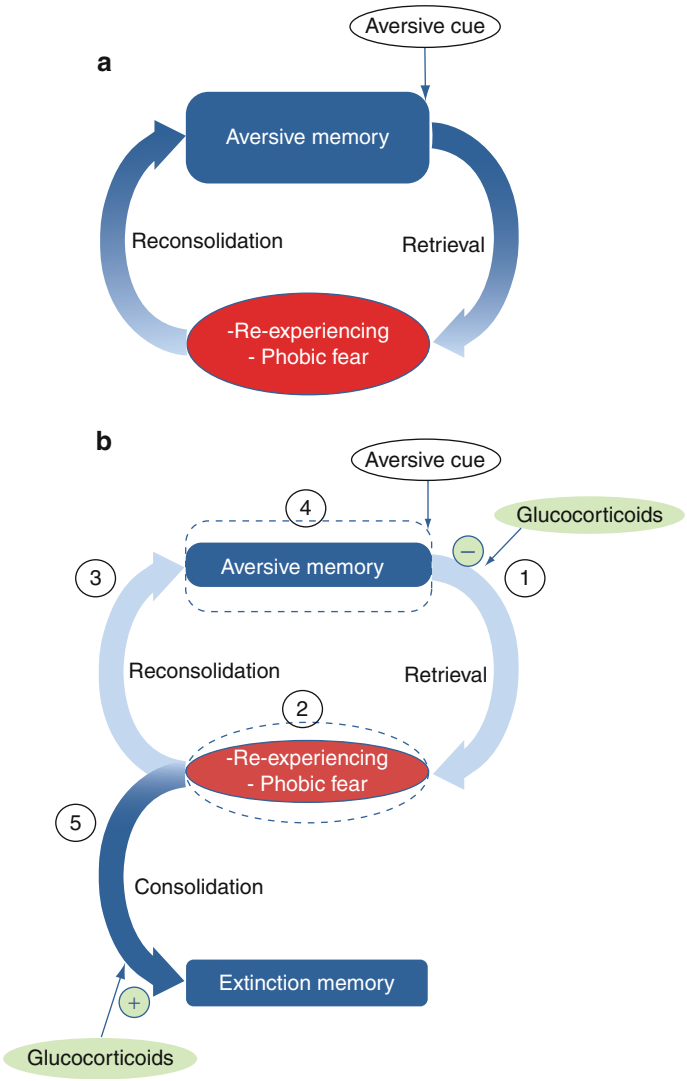

Fig. 5.4 Model on the role of glucocorticoids in the reduction of aversive (traumatic) memory. (**a**) Excessive retrieval of aversive memories causes re-experiencing symptoms in PTSD. Reconsolidation of such aversive experiences further cements the aversive memory trace. (**b**) Glucocorticoid-induced reduction of the aversive memory trace. By inhibiting memory retrieval, glucocorticoids partly interrupt this vicious cycle of retrieving (1), re-experiencing (2), and reconsolidating (3) aversive memories, which leads to a weakening of the aversive memory trace (4). Furthermore, because the aversive cue is no longer followed by the usual aversive memory retrieval and related clinical symptoms, the cue becomes associated with a non-aversive experience, which is stored as extinction memory (5). Based on the findings of animal studies, glucocorticoids are likely to enhance long-term consolidation of extinction memory (see text for details) (Reprinted from de Quervain et al. 2009)

post-retrieval effects of glucocorticoids on memory are of transient nature and are reversed by a reminder [but see Wang et al. (2008)], which should not occur after inhibited reconsolidation. Although the data currently available rather speak for a facilitating effect of glucocorticoids on memory extinction, it is possible that, perhaps under certain conditions, glucocorticoids may also inhibit memory reconsolidation processes.

5.4 Conclusion

In this chapter, we have reviewed evidence from both animal and human studies indicating that glucocorticoids enhance memory consolidation, but impair memory retrieval and working memory (Fig. 5.3). Importantly, these hormone effects depend on emotional arousal-induced activation of noradrenergic transmission within the BLA and on interactions of the BLA with other brain regions, such as the hippocampus and neocortex. Therefore, glucocorticoids, via BLA activation, can modulate memory processes of many different kinds of emotionally arousing experiences.

Enhanced consolidation for emotionally arousing information is an adaptive mechanism, which helps us to retain important information. Reduced memory retrieval and working memory should not a priori be regarded as maladaptive as they support this process of retaining important information. In addition, the reduction of memory retrieval may aid in suppressing behaviors that are no more relevant or even maladaptive. This mechanism is especially important in more chronic situations when the organism is forced to adapt to a changed environment (e.g., during space flight). Under such conditions, also the facilitating effects of glucocorticoids on extinction processes represent an adaptive response, which helps the organism to deal with stressful events (De Kloet et al. 1999; McEwen 1998).

Because emotionally aversive memories play an important role in the development and symptomatology of stress-related (anxiety) disorders, we aimed to translate the basic findings on the effects of glucocorticoids on emotional memory in animals and healthy humans to clinical conditions. Specifically, the findings, which indicated that glucocorticoids reduce memory retrieval and enhance extinction of emotional memories, led us to hypothesize that these stress hormones might be useful in the treatment of anxiety disorders. Clinical studies and studies in animal models of acquired fear indicate that glucocorticoid treatment indeed reduces the retrieval of traumatic memories and enhances extinction processes. These dual actions of glucocorticoids seem to be especially suited for the treatment of acquired fear. By inhibiting memory retrieval, glucocorticoids may partly interrupt the vicious cycle of spontaneous retrieving, re-experiencing, and (re)consolidating aversive memories, in patients on earth, as in space crew members who have undergone an unpredicted, traumatic emergency situation.

More research is needed to better understand the molecular underpinnings of glucocorticoid actions on different memory processes as well as the role of (epi)genetic differences across individuals (de Quervain et al. 2007b). Such research might further

promote the understanding of why some individuals become vulnerable to anxiety disorders, whereas others are resilient or even gain strength from stressful experiences. This knowledge might be also of considerable importance when selecting crew members for a long-duration mission for space exploration. Moreover, because cognitive dysfunctions, stress disorders, and the effects of glucocorticoids are linked to downstream immune modulation, a more integrative view is needed, also with regard to possible countermeasures. Finally, as stress and stress hormones have a large impact on specific memory functions and space flight conditions are associated with many stressors, such as microgravity, sleep deprivation, pain, variable oxygenation status, radiation, and confinement, research has been initiated to investigate these specific cognitive functions in space flight conditions together with the psycho-neuroendocrine and immune status.

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The Autonomic Nervous System

6

Nicola Montano, Eleonora Tobaldini, and Alberto Porta

6.1 Introduction

The autonomic nervous system (ANS) is the branch of the nervous system that controls the visceral functions in order to maintain the "homeostasis/homeodynamic" state of the body, being an interface between body, central nervous system (CNS), and the external stimuli (Malliani 2000).

The ANS can be divided into two subsystems, the sympathetic (SNS) and the parasympathetic (PNS) branches. The autonomic nervous system plays a key role in the regulation of several body functions like control of cardiovascular function, gastrointestinal, pulmonary, skin, and genitourinary and immune control, just to cite the most important, that are all essential in order to maintain the physiological body functions. Moreover, the ANS is very sensitive to react upon environmental challenges / stressor to make the body adaptable to the environment (Fig. 6.1).

Despite the widely used definition of "autonomic," a lot of evidences strongly support the hypothesis of very complex and integrated control mechanisms operating at different central and peripheral levels coordinating the function of ANS (Montano et al. 2009).

Just considering this latter point, it has also been widely demonstrated that several pathological conditions (e.g., cardiovascular diseases such as hypertension, heart failure, and myocardial infarction) are characterized by an impaired autonomic control, consisting of a chronic sympathetic overactivity, due to both afferent and

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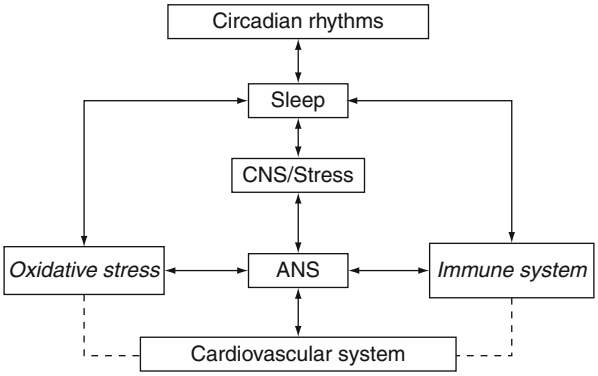

Fig. 6.1 Schematic representation of the central role played by autonomic nervous system (ANS) in being modulated/modulating by stress system in the central nervous system (CNS). This is in turn affected by sleep, which is under the major influence of circadian rhythms. Changes in ANS induced by stress system and/or sleep may affect cardiovascular system not only directly, but also increasing oxidative stress and modifying immune responses. As this relationship is bidirectional, primary changes in oxidative stress and immune responses also may affect ANS and, moving upstream, stress responses and sleep

efferent pathways activation, characterized by altered responses to stressors stimuli (Lombardi 1986; Guzzetti et al. 1988; Pagani and Lucini 2001).

Therefore assessment of ANS regulation is fundamental in order to identify the pathological basis of such diseases (cardiovascular and non-cardiovascular) and can also be an important target for pharmacological therapy (Malliani 2000; Montano et al. 2009).

Recently, it has been demonstrated that the ANS plays an important role not only in the regulation of vegetative state, but also in modulating immune system responses (Tracey 2009), metabolism, inflammation (Sternberg 2006; Grassi et al. 2007), thus suggesting a complex integrative role of ANS at different control levels.

In conclusion, it is clear how important is the assessment of ANS in physiological and pathological situations as modifications of the control mechanisms regulating autonomic functions under various conditions of stress challenges.

6.2 Methodologies

Since many years ago, several invasive and noninvasive different techniques have been developed for the analysis of ANS; however, the attempt to find out a noninvasive, reliable, and reproducible technique is still challenging.

Considering all the methods developed for the ANS evaluation, it is possible to identify three main groups of techniques: (a) humoral measures of sympathetic activity based on the quantification of catecholamines; (b) direct assessment of muscle sympathetic nerve activity (MSNA) by means of microneurographic techniques; and (c) indirect measures of sympathetic and parasympathetic modulations derived from the analysis of heart rate variability analysis (HRV).

6.2.1 Catecholamines and Muscle Sympathetic Nerve Activity

For many years, the levels of plasma and urinary catecholamines (epinephrine and norepinephrine) have been considered as a reliable index of sympathetic activity. In 1983, Goldstein et al. reported that hypertensive subjects had higher plasma catecholamines levels compared to healthy subjects (Goldstein et al. 1983). Several factors may influence catecholamine levels and kinetics and have been identified as possible confound elements contributing to the variability among subjects. Moreover, because the plasma levels of catecholamines suggest general and non-organ-specific changes in sympathetic control, more specific regional measures of catecholamines have been developed. In fact, measures of the local rates of noradrenaline release could allow the assessment of organ-specific sympathetic nervous system activity, as the cardiac catecholamines spill over (Esler 1993).

Sympathetic activity could also be directly recorded in humans, using the microneurography techniques (Valbo et al. 1979). The direct recording of sympathetic nerve activity directed to skeletal muscles (MSNA) and skin (SSNA) has provided important information regarding cardiovascular control reflexes in several physiological and pathological conditions (exercise, response to orthostatic stress, cardiovascular and metabolic diseases such as hypertension, heart failure, obstructive sleep apnea etc.) (Narkiewicz and Somers 2003; Wallin et al. 2007; Kato et al. 2009). Although these techniques are more reliable than the catecholamine measurements in assessing the sympathetic function, the limitation that they are minimally invasive makes this approach unsuitable for studies on large populations (Montano et al. 2009).

Another key observation is the fact that all the techniques mentioned above are capable of providing information on sympathetic branch; they cannot give any information regarding the parasympathetic activity. These two limitations, the invasivity and the lack of information on parasympathetic nervous system, lead to the development of noninvasive techniques capable of providing information on the sympatho-vagal balance and on the rhythmical oscillations on the heart period and arterial pressure variability in the time and frequency domain.

6.2.2 Heart Rate Variability Analysis

More than 40 years ago, Lee and Hon (1965) described, in fetal distress, alterations in interbeat intervals preceding evident changes in heart rate itself.

Some years later, based on the hypothesis that heart rate and blood pressure exhibit beat-to-beat oscillations around the main values and that the identification of these rhythmical components can provide reliable information on sympathetic and parasympathetic modulation of heart rate and blood pressure variability (Pagani et al. 1986). Sayers and coworkers (Hyndman et al. 1971; Sayers 1973) introduced the idea of a computational analysis of the variability of heart rate (heart rate variability, HRV) as a reliable tool capable of investigating sympathetic and parasympathetic modulations. Kleiger found further evidence of a strong clinical relevance of

HRV in cardiac patients in a pioneering study, where HRV was found to be an independent predictor of mortality after acute myocardial infarction (Kleiger 1987; Malik 1989) provided the evidence of the clinical relevance of this technique.

Differently from the time domain measures, HRV analysis is based on the idea that each time series, including the RR interval time series (tachograms) and the systolic and diastolic arterial pressure time series (systograms and diastograms), can be considered as the sum of different rhythmical oscillations, characterized by specific frequency and amplitude. It has been clearly shown that the assessment of these rhythmical components on cardiovascular signals provides reliable information on the ANS function in healthy people and the diseased (Akselrod et al. 1981; Malliani et al. 1991).

In the last few decades, intensive research in this field allowed to better understand the physiological interaction between sympathetic and parasympathetic efferent pathways, the sympatho-sympathetic excitatory reflexes (Malliani et al. 2002), and the alteration of the sympatho-vagal balance in pathological conditions that form as basis for cardiovascular diseases.

6.2.2.1 Time-Domain Analysis

Time domain analysis of HRV is based on the evaluation of the RR intervals. After the QRS complex detection, it is possible to determine the normal-to-normal (NN) intervals, consisting of all intervals between adjacent QRS complexes from sinus node depolarizations. The simplest time domain analysis includes mean NN interval, mean heart rate, the difference between the longest and shortest NN interval or variations of instantaneous heart rate due to respiration, Valsalva maneuver and drug infusion (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology 1996).
For more technical details, see Chap. 20.

6.2.2.2 Frequency Domain Analysis

Several methods have been validated for the spectral analysis of HRV and those can be divided into parametric and nonparametric methods. The nonparametric methods use a more simple algorithm (usually a fast Fourier transform, FFT) and the process speed is higher, while the parametric methods allow the identification of spectral components independent of preselected frequency bands, automatic calculation of low- and high-frequency components, and the possibility of precise estimation of spectrum density on small samples (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology 1996).

From the time series, the algorithm is capable of distinguishing three main spectral components: very low frequency component (VLF), frequency band below 0.04 Hz, low-frequency component (LF), centered around 0.1 Hz (0.04–0.15 Hz), marker of sympathetic modulation, and a high-frequency component (HF), synchronous with respiration, marker of vagal modulation. Each oscillation can be described by a specific frequency band and amplitude that is expressed in absolute values of power (milliseconds squared) and in normalized units (nu), which represent the relative value of each power component in proportion to the total power minus the VLF component.

As for the previous paragraph, see Chap. 19 for more technical details and explanations.

6.2.2.3 Nonlinear Analysis of HRV

In the past few years, a growing interest has been focused on the investigation of nonlinear dynamics of HRV and on the evaluation of the complexity of the cardiovascular control of HRV and blood pressure variability.

The hypothesis that ANS control is based on nonlinear dynamics and on the interaction of different subsystems that act at several time scales leads to the growing interest in providing nonlinear analyses and complexity measures of cardiovascular ANS control in physiological and pathological states (Goldberger and West 1987; Goldberger et al. 1988; Kaplan et al. 1991; Porta et al. 2007; Maestri et al. 2007; Voss et al. 2009; Huikuri et al. 2009).

In a recent review, Voss et al. (2009) suggested that the nonlinear dynamics and complexity of a biological system can be due to three main components: (1) different subsystem acting with feedback interactions to adapt to internal and external stimuli, (2) the adaptation of a subsystem to changed conditions (as in pathological conditions or during aging process), (3) when a subsystem failed, the others operate to compensate the lacking control mechanisms.

Three main families of nonlinear and complexity analyses can be identified: (1) fractal measures, such as Power-law correlation and detrended fluctuation analysis (DFA); (2) symbolic dynamics, and (3) complexity measures, such as Sample Entropy, Approximate Entropy, Multiscale Entropy, Shannon Entropy, Conditional Entropy, and Corrected Conditional Entropy.

Detrend Fluctuation Analysis

Detrended fluctuations analysis (DFA) was introduced by Peng et al. 1995 and it is based on a modified random walk analysis applied to physiological time series (Peng et al. 1995). It quantifies the fractal correlation properties in non-stationary time series and it usually estimates a short-term fractal scaling exponent $\alpha 1$ and a long-term scaling exponent $\alpha 2$, to identify the fluctuations on multi-length time scales. $\alpha 1$ and $\alpha 2$ describe, respectively, the short- and the long-term fractal-like scaling properties of the heart period fluctuations.

Technically, the time series is divided into windows of the same size and the variability is analyzed in relation to local trend in each window and the process is repeated for all different window sizes. A scaling exponent value of approximately 1 corresponds to 1/f fluctuations.

In healthy subjects, the scaling exponent is approximately 1, thus suggesting a fractal-like behavior, while in pathological conditions, this index is reduced, suggesting a loss of fractal-like heart period dynamics (Mäkikallio et al. 2002; Huikuri et al. 2009; Voss et al. 1995, 2009). This technique has been applied for the identification of high-risk cardiac patients: It has been shown by Makikallio et al. (Mäkikallio et al. 1997; Huikuri et al. 2000) that a reduction of $\alpha 1$ is a powerful predictor of mortality and that this reduction is associated with vulnerability to ventricular tachycardia, ventricular fibrillation, and arrhythmic death.

Symbolic Analysis

In the last few years, a new method called symbolic analysis (SA) has been implemented by several authors (Porta et al. 2001, 2007; Voss et al. 2009) as a nonlinear tool capable of providing information on autonomic cardiovascular control. SA is based on the conversion of a series into a sequence of symbols suitable for the description of sympathetic and parasympathetic modulation of HR and BPV. Briefly, it is based on the transformation of heart period variability series into a sequence of symbols and the construction of patterns (i.e., words). The complexity of the data time series is determined from the distribution of the words using different mathematical methods (Voss et al. 1995; Wessel et al. 1998, 2000; Porta et al. 2007a–d).

In the method proposed by Porta et al., the full range of sequences is spread over six levels and patterns of three beats length are constructed. All the patterns are then grouped, without any loss, into four families: the 0V family (patterns with no variations), 1V family (patterns with one variation), 2LV family (patterns with two like variations), and 2UV family (patterns with two unlike variations) (Porta et al. 2007a). The rate of occurrence, expressed as a percentage, is then evaluated. The application of this method to evaluate cardiac autonomic control of HRV revealed that the family of 0V pattern is marker of sympathetic modulation, while the 2UV family is a marker of vagal modulation. Compared to spectral analysis, this method has some advantages: SA is a nonlinear method, it is independent of the definition of the a priori frequency bands of the heart period oscillations and it is more reliable than spectral analysis in evaluating ANS control during co-activation of the two branches. It has been shown that SA is capable of tracking the progressive changes of sympathetic modulation occurring during graded head-up tilt test better than more classic spectral analysis (Porta et al. 2007d). The clinical application of this technique allows the identification of high-risk patients after acute myocardial infarction (Voss et al. 1995) and a better predictor of high risk for arrhythmias in post-myocardial infarction patients.

Entropy Measures

In order to assess the regularity (or, its opposite, the irregularity) of the heart period and blood pressure oscillations, in the last few years, several entropy measures have been developed. Complexity analysis can provide the quantification of different aspects of the cardiovascular control mainly related to the organization of different subsystems that cooperate in order to regulate the physiological and pathological responses of cardiovascular ANS. Complexity is measured by evaluating the amount of information carried by a series, i.e., larger the information, greater the complexity (Porta et al. 2001). For the evaluation of complexity of heart period variability, several indices have been developed and applied in physiological and pathological conditions. In physiological conditions, a maneuver known to induce a gradual increase of sympathetic modulation, i.e., the head-up tilt test, caused a reduction of complexity of heart period variability. Moreover, several studies have shown that aging and pathological processes are associated with a decrease in complexity of autonomic cardiovascular control.

Approximate Entropy (ApE) and Sample Entropy (SE)

ApE can be considered an index of the overall complexity of a time series (Pincus 1991). It measures the unpredictability of fluctuations in a time series, quantifying the probability that patterns will remain similar for successive incremental comparison (Pincus and Goldberger 1994). The higher is the ApE, the higher is the complexity of the time series, the lower is the ApE, the lower is the complexity of the time series (i.e., the time series is more predictable because it contains many repetitive patterns).

SE is an improvement of ApE and it quantifies the conditional probability that two sequences of data points similar to each other will remain similar when one consecutive point is included (Voss et al. 2009).

Shannon Entropy (ShE)

ShE assesses the complexity of the distribution of the patterns of length L. ShE is an index that describes the shape of the distribution of heart periods: ShE is large if the distribution is flat (all the patterns are identically distributed and the pattern distribution carries the maximum amount of information). On the contrary, ShE is small if there is a subset of patterns that is more likely, while others are missing or infrequent (as it happens in a Gaussian distribution) (Porta et al. 2007).

Conditional Entropy (CE) and Corrected Conditional Entropy (CCE)

CE is a measure of the amount of information carried by the current RR sample when the previous values are known. In other words, it represents the difficulty in predicting future values of RR based on past values of the same series. The CE is 0 when the knowledge of the previous values is helpful in predicting the future values while it is equal to ShE when the knowledge of past values of RR is not helpful to reduce the uncertainty of future RR values. The CCE, proposed by Porta et al. (2001), decreased to 0 only when RR was completely predictable, showed the maximum value when RR is completely unpredictable and showed a minimum when the knowledge of past values is helpful in reducing the uncertainty associated with future RR values.

6.3 Mirroring the Interaction of Stress and the Autonomic Nervous System Through Cardiovascular Read-Out Parameters

The pivotal role of the autonomic nervous system in regulating instantaneous cardiovascular responses to everyday stressors justifies the major interest of investigating variations of cardiovascular autonomic regulatory mechanisms on earth and in space.

Neural control of cardiac function and circulation is mainly regulated by the interaction between sympathetic and parasympathetic efferent pathways. Usually, physiological regulation of cardiovascular control and responses to external stimuli is based on the concept of sympatho-vagal balance: The system is based on a reciprocal response of the two pathways with an activation of the sympathetic drive accompanied by an inhibition of the vagal outflow and vice versa (Malliani 2000). This bal-

ance allows the autonomic cardiovascular system to maintain an homeostatic condition in response to internal and external stimuli (exercise, response to orthostatic challenge, stressors, etc.) and oscillates from a states of quite, when negative feedback reflexes are predominant, to states of excitation (for physical or mental stress) characterized by central excitatory mechanisms reinforced by peripheral positive feedback reflexes (Malliani et al. 1991; Zucker 1996; Legramante et al. 1999; Malliani 2000; Montano et al. 2009).

However, particular physiological situations such as exercise, cold face immersion, and apneas (Paton et al. 2005) but also pathophysiological situations, such as the period immediately preceding ventricular major arrhythmias (Guzzetti et al. 2005), are characterized by a co-activation of the two subsystems, thus leading from one side to visceral adaptation to stressful conditions but from the other pathological conditions.

While acute activation of the sympathetic nervous system subserves fundamental regulatory functions and it is necessary to human phylogenesis (exemplified by the "fight or flight" concept), chronic sympathetic activation as occurring during stressful conditions (physical and or psychological) may result in profound alterations of neural visceral regulatory functions, leading from functional disturbances up to the development of cardiovascular and non-cardiovascular diseases. Short- and long-term spaceflight may challenge cardiovascular autonomic control; thus, relevant information about autonomic mechanisms can be inferred from the analysis of cardiovascular variability changes.

6.3.1 Cardiovascular Autonomic Control During Immobilization Stress (Head-down Bed Rest)

The Head-Down Bed Rest (HDBR) is a stress-full condition consisting of maintaining subjects strictly in bed, with −6° of head down and it has been widely used in experimental physiology as a simulation of microgravity environment characteristic of space flights on various organ-systems (see also Chap. 31). The short-term and long-term HDBR allowed researchers to investigate the effect of microgravity on autonomic cardiovascular functions in terms of heart rate and blood pressure variability, baroreflex responses, plasma and urinary catecholamines levels mimicking short- and long-term space flights. The major interest in studying HDBR-related changes in autonomic control is the possibility to mimic the weightlessness and microgravity that are known to cause orthostatic intolerance and the so called deconditioning phenomenon, experienced by astronauts after landing, characterized by the reduced orthostatic tolerance, pre-syncope, and syncope.

Several studies investigated the autonomic cardiovascular responses of cardiovascular parameters, heart rate variability indices, and baroreflex sensitivity during short-term and long-term HDBR and the recovery period.

The main effects of short-term HDBR exposure is an increase of HR and a reduction of the total variability of heart period and of parasympathetic modulation and an impairment of baroreflex function (Hirayanagi et al. 2004; Traon et al. 1998;

Crandall et al. 1994). In fact, a reduction of the high-frequency component, HF, marker of vagal modulation, has been observed during short-term HDBR in the late phase of the test and during the early recovery period (Hirayanagi et al. 2004), as well as a reduction in sympathetic modulation more evident in the late phase of the test (Traon et al. 1998).

The data on long-term HDBR (60–120 days) are more debated; for example, the long-term exposure to HDBR has been shown to both increase (Kamiya et al. 2003) and decrease the muscle sympathetic nerve activity (Ferretti et al. 2009). As to cardiovascular parameters, heart rate was significantly higher after 60 and 120 days of HDBR while blood pressure remained unchanged during the entire period (Kamiya et al. 1999); considering HRV and the baroreflex function, a significant decrease in HRV and baroreflex sensitivity both in the late phase of HDBR and the early phase of recovery period has been observed; however, the HDBR did not alter the rhythmical oscillatory components of heart period and blood pressure variability (Ferretti et al. 2009). Interestingly, it has been shown that during a 24-h HDBR the heart period variability indices and the baroreflex sensitivity expressed a relevant circadian variation, characterized by an increase of total power and high-frequency component during the evening (Hartikainen et al. 1993).

In addition to the standard frequency domain approach to heart rate variability, the nonlinear analysis of HRV after HDBR revealed a reduction in the complexity of heart period variability, underlying the important effects of the microgravity and weightlessness on autonomic cardiovascular control and organization (Goldberger et al. 1994).

All these results support the hypothesis that a decrease in HRV, thus an increase in cardiovascular sympathetic modulation**,** associated with other factors, such as hypovolemia and impairment of endothelium-dependent functions at the microcirculation level, could be responsible for the deconditioning effect experienced after weightlessness predisposing subjects to postural hypotension and reduced ability to react to orthostatic stimuli (Kamiya et al. 2003; Coupé et al. 2009).

6.3.2 Cardiovascular Autonomic Control During Acute Gravitational Stress (Parabolic Flight)

Parabolic flights are used to create and reproduce short periods of changing gravity, within a range of 0–1.8 Gz, with 1 Gz equal to 9.81 m/s^2. A standard parabolic flight can be divided into five different phases: 1 Gz (before and after each parabola), hypergravity during the ascending leg of the parabola, microgravity at the apex of parabola and hypergravity during the descending leg of parabola.

Several studies have investigated the hemodynamic and cardiovascular changes during and after parabolic flights, that have been used to simulate hypergravity and microgravity characteristics of space missions.

During the microgravity, there is a redistribution of blood toward the upper body that is capable of stimulating baroreceptors (Lipnicki 2009) and left atrial diameter and transmural central venous pressure increase (Videbaek and Norsk 1997), as

well as intrathoracic blood volume and systemic blood pressure (Iwase et al. 1999). However, in supine position, microgravity induced a decrease of mean arterial pressure and an increase of left atrial diameter, while during 1 Gz conditions left atrial diameter increased (Pump et al. 1999).

As to HRV, several studies revealed a higher vagal modulation in microgravity compared to hypergravity in standing position; however, no significant differences in terms of spectral parameters were found in supine position between the different flight phases (Seps et al. 2002; Beckers et al. 2003). The assessment of sympathetic nervous system using the MSNA technique revealed that during parabolic flights, with subjects seated, MSNA was increased under hypergravity before microgravity entry and then suppressed at the onset of microgravity in response to loading of the cardiopulmonary volume receptors (Iwase et al. 1998, 1999).

The hemodynamic and cardiovascular responses in the gravitational changes during parabolic flights may be responsible for the syndromes of orthostatic intolerance that mimic the symptoms after space flight, even after short parabolic flights (Schlegel et al. 2001).

6.3.3 Cardiovascular Autonomic Control During Space Flights

Several studies described that autonomic control of cardiovascular functions is impaired during space flights, representing a risk factor for cardiovascular diseases for the astronauts.

It has been shown that a short exposure to microgravity, as during the short-term space flights, is capable of inducing a reduction of orthostatic tolerance and increased plasma norepinephrine and epinephrine levels (Fritsch-Yelle et al. 1994), an increase of baroreflex sensitivity in the early phase of microgravity, reducing the respiratory modulation of heart rate and decreasing cardiac baroreflex gain with no effects on arterial blood pressure during post-flights period (Verheyden et al. 2007). The early exposure to microgravity induced a decrease of HR both in supine and standing position; diastolic pressure and premature ventricular contractions all were significantly reduced in flight (Fritsch-Yelle et al. 1996) while overall variability, LF/HF and respiratory sinus arrhythmia amplitude and phase were similar to pre-flight values (Migeotte et al. 2003).

Compared to Earth, systolic arterial pressure and HR tended to be higher in space with an impairment of vagal baroreflex control, suggesting the idea that microgravity exposure induces a prevalence of sympathetic and a decrease of parasympathetic cardiovascular control (Eckberg et al. 2010). The analysis of HRV during long-term exposure to microgravity revealed an alteration of the rhythmic oscillatory components with the evidence of a super-slow wave oscillation, marker of ultradian rhythms, supporting the hypothesis of a new setting point of cardiac autonomic regulation (Baevsky et al. 1998). Similarly, MSNA tended to be higher when recorded in astronauts during spaceflight than on Earth (Eckberg and Neurolab Autonomic Nervous System Team 2003).

In the last few years, an increasing interest has also been focused on the autonomic changes in space flights during sleep. The daily mean systolic blood pressure and heart rate during space flight were similar to the pre-flight values, but during sleep, while no differences from pre-flights values have been observed in HR, an increase to over pre-flight values of systolic arterial pressure was described (Shiraishi et al. 2004). Comparing the different sleep stages, a more pronounced decrease of HR was observed during non-REM than for REM sleep (Gundel et al. 1999), thus suggesting a possible increase of parasympathetic dominance of cardiac rhythms in space.

One of the major problems of astronauts who return to Earth after space flights is the possibility to experience orthostatic intolerance and, rarely, syncope, for a deconditioning effect of microgravity on autonomic cardiac control. This is the reason why several studies investigated the autonomic control and the response to orthostatic challenge after space missions (Eckberg et al. 2010). It has been observed that long-term space flights (9 months) have been able to reduce the total power of HRV and to increase the systolic pressure variability, suggesting a reduction of the cardiac vagal control and the baroreflex gain (Cooke et al. 2000).

Studying the autonomic response to orthostatism, it has been demonstrated that astronauts who were not able to finish the orthostatic stress (tilt test) had a lower systolic arterial pressure at rest in pre-flight condition while, at the end of the tilt test, HR and systolic arterial pressure were lower in nonfinisher than finishers (Sigaudo-Roussel et al. 2002). These subjects also exhibited low vascular resistance and smaller response to phenilephrine before and after flights and low norepinephrine release during orthostatic challenge after landing. Similarly, the MSNA response to orthostatic challenge was preserved in the finishers (without orthostatic intolerance) but it was reduced in those who experienced orthostatic intolerance, supporting the hypothesis of an impairment of baroreflex control (Mano 2005).

These data suggested that the orthostatic intolerance after space flights could be due to a decreased number of alpha-1 adrenergic receptor responsiveness before the flight and a remodeling of the central nervous system that occurs during space flight (Meck et al. 2004).

6.4 Stress, Autonomic Nervous Systems and Immune Regulation

The pivotal role of the autonomic nervous system (ANS) in regulating instantaneous bodily responses to everyday stressors and especially in space has been described exemplarily on the interaction between the ANS and the cardiovascular systems as above, showing the cardiovascular changes as a critical indicator of the role of the ANS in the regulation of the human stress-homeostasis. As stated above, in neuroautonomic terms “stress” may be equated to “sympathetic overactivity” that is a condition in which there is no more balance and the parasympathetic modulation is shut down. Mounting evidence suggests that an imbalance of the ANS and its

respective hormones (e.g., catecholamines) is affecting immune responses. For instance, with some evidence that the sympathetic increasing and the vagus decreasing the release of pro-inflammatory cytokines with relevance to diseases (Bellinger et al. 2008). Moreover, it has been described that space flight–associated stressful situations can result in a sympathetic and/or glucocorticoid-mediated immune downregulation (Stowe et al. 2003) which was also associated with reactivation of herpes virus (Stowe et al. 2001). In brief, stressful conditions of psychological or physical nature do modulate key functions of the hosts' immune responses by neurohumoral, catecholaminergic- and glucocorticoid-system together with neural pathways to control the host's immune homeostasis (Tracey 2002, 2009).

It appears likely that ANS alterations in space may impinge also upon homeostatic immune mechanisms, thereby modulating key function of innate and adaptive immune responses in space (please see Fig. 6.1 and Chaps. 9–13 of this volume).

6.5 Summary

The autonomic nervous system plays a crucial role as interface between visceral function and physical/emotional stress challenges. Once regarded only as a source of pure efferent motor output, it is now well-established that it is a complex system based on the integration of several reflexes having both negative and positive feedback characteristics, continuously balancing each other. This reflex activity is directed both upstream, impinging upon CNS structures related to sleep–wake cycle and activating the arousal system, as well as downstream, regulating visceral (cardiovascular, gastrointestinal and genitourinary) functions. Thus, changes in ANS activity may affect sleep and cortical responses to stress and vice versa. Interestingly, it has recently observed that ANS is also able not only to be modulated, but also to modulate immune response, thus strengthening the importance of ANS as a possible target system to elaborate countermeasures to better cope with stressful conditions, such as space environment.

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Circadian Rhythm and Stress

7

Mathias Steinach and Hanns-Christian Gunga

7.1 Circadian Rhythm

7.1.1 Definition and Regulation

Rhythmicity of physiological variables is a ubiquitous phenomenon in living systems (Aschoff and Wever 1962a). Several different rhythms appear to exist, from long wavelength rhythms like the monthly menstrual cycle to circadian rhythms of many parameters. Several early works of research have described diurnal changes and rhythmicities in biological phenomena like the pulse rate, respiratory gas exchange, or body temperature, exhibiting the peak temperature at the early evening (6 p.m.) and a nadir during the late night and early morning (4 a.m.) (Gierse 1842). This rhythmicity seemed to be innate, as it was unaffected by fasting or physical activity. Also it was shown that isolation from external time cues (zeitgeber) like most notably sun light, but also social interaction, left the circadian rhythm intact, even though its phase period appeared to become elongated (Aschoff and Wever 1962b). It was concluded that there is an endogenous origin of the human circadian rhythm. Since then, several similar experiments have revealed an endogenous rhythmicity of approximately 24.5 h, when no external time cues are present (Halberg et al. 1965).

The circadian rhythm is created by a pacemaker system located in the suprachiasmatic nucleus of the anterior hypothalamus (SCN) (Mistlberger and Rusak 1989), which coordinates molecular clocks in each body cell through the control of body temperature, hormone concentrations, and behavior as well as through a transcription/translation feedback loop of clock-related genes (e.g., Per1-2, Cry1-2) influenced by a protein dimer (BMAL1-CLOCK – brain and muscle ARNT-like protein 1 and circadian locomotor output cycles kaput) in all peripheral cells that are similarly regulated by hormones and cytokines (Levi et al. 2008) (Fig. 7.1). A model of

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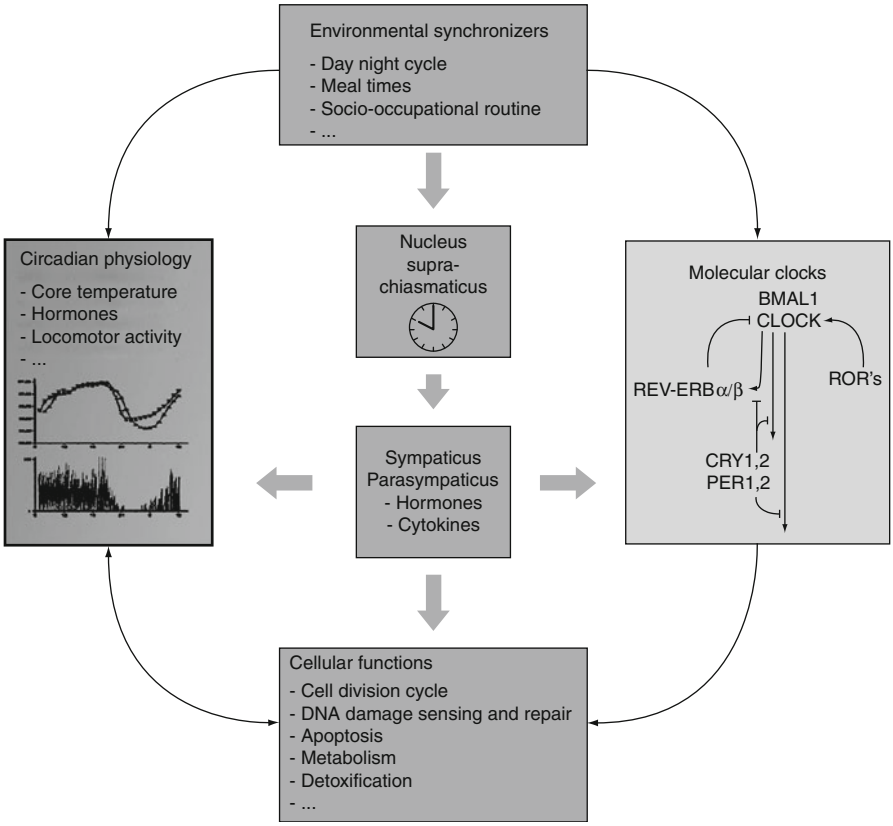

Fig. 7.1 Schematic view of the circadian system. The periods of the suprachiasmatic nuclei are calibrated by the day–night cycle and other environmental synchronizers. The suprachiasmatic nuclei control cellular rhythmicity through their influence on hormonal alterations, locomotor activity, and the body core temperature. Other pathways involve the sympathetic and parasympathetic systems as well as cytokines like TGFα and EGF. Molecular clocks in all peripheral cells are controlled by these influences involving transcription/translation feedback loops, in which the BMAL1:CLOCK protein dimer plays a central role. The molecular clocks in turn influence cellular functions and eventually affect circadian physiology (After Filipski et al. 2009) BMAL1: Brain And Muscle Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT)-Like, CLOCK: Circadian Locomotor Output Cycles Kaput, REV-ERBa/b: BMAL1- and CLOCK-regulating transcriptions factors, also known as: Nuclear Receptor Subfamily 1, Group D, Member 1 (NR1D1), ROR: RAR-related Orphan Receptor also known as Nuclear Receptor Subfamily 1, Group F, Member 1 (NR1F1), CRY: Cryptochrome, PER: Period

this system consists of an SCN oscillator generating a rhythm of approximately 24 h, with input pathways responsible for synchronizing the internal system with external stimuli like the natural light–dark cycle, and output rhythms that are regulated by the pacemaker; the synchronization process that adjusts the internal rhythm to the external zeitgeber is called entrainment (Moore-Ede et al. 1982). As light reaches the retina, this information is transmitted to the circadian system via a direct pathway (Fig. 7.2), the retinohypothalamic tract (RHT) and via an indirect pathway

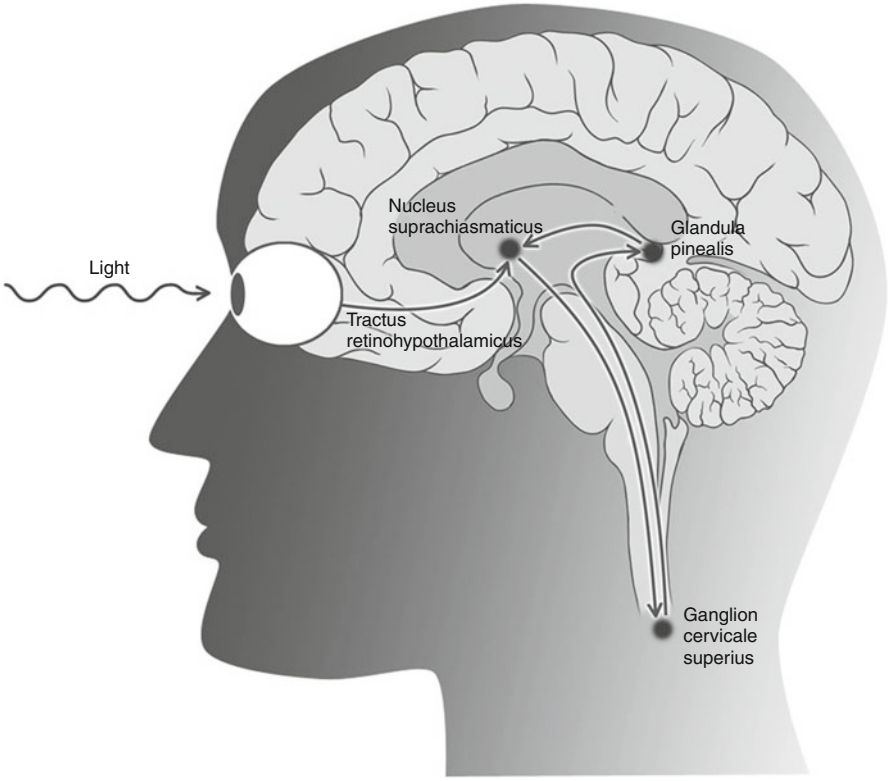

Fig. 7.2 Schematic view of the neural pathways on the effects of light influence on the circadian system. Light information is routed to the suprachiasmatic nuclei (SCN). From there, the inhibition signals reach the Glandula pinealis via the Ganglion cervicale superius. This inhibition disappears in the absence of light (After Reid and Zee 2009)

through the intergeniculate leaflet (IGL) (Harrington 1997). It has been suggested that melanopsin-containing retinal ganglion cells, which appear to be neither rods or cones, are the primary circadian photoreceptors in the retina (Ruby et al. 2002), exhibit the most sensitivity to wavelengths of around 460 nm (Warman et al. 2003) and it as has been shown that light of this wavelength induces the greatest changes in circadian light–induced phase shifts and melatonin suppression. Melatonin, a tryptophan metabolite, is secreted by the pineal gland which has afferent and efferent connections to other parts of the circadian system. The pineal gland is innervated through the superior cervical ganglia of the sympathicus, which receives input from the SCN. Melatonin from the pineal gland in turn affects the SCN as it can alter the timing of the circadian rhythms and helps to promote sleep as it inhibits the neurons located in the SCN, thus creating a sleep-permissive condition (Liu et al. 1997).

A model of the circadian clock consists of an endogenous circadian process (process C) and a homeostatic process (process S) and environmental factors. The endogenous process facilitates wakefulness during the day and the consolidation of sleep during the night (Zulley et al. 1981). In addition, the homeostatic process

accumulates as a function of prior wakefulness, so that the drive for sleep continues to rise proportionally until it is reduced through sleep, momentarily the only known means to reduce the sleep propensity of the homeostatic process (Dijk et al. 1990).

The regulation process is also associated with thermoregulation, as was shown that sleep is usually initiated when the body temperature begins to fall and the awakening is triggered after the body temperature begins to rise again. A hypothermic effect of melatonin that affects sleep propensity has been proved (Krauchi et al. 2000). The induction of sleep through peripheral heat loss via vasodilatation followed by a decline of body core temperature further supports the suggested relationship between the sleep–wake cycle and thermoregulation (see also Chap. 23).

The highly regulated system of the circadian rhythm can be disturbed due to a misalignment between the endogenous circadian phase and the external 24-h-based social and physical environment. Also stressful events or conditions that cause alterations in the regulation of associated mediators like stress hormones can influence the sleep–wake cycle, as described below.

To the end of the aforementioned alteration during stress – especially with regard to hormonal changes like in the HPA axis with their influence on alertness and vigilance – it becomes obvious that stressful events can have a direct impact on the sleeping pattern and ultimately the circadian rhythm of humans exposed to stress. The following paragraphs shall illustrate these relationships and different forms of circadian misalignment in stressfull conditions more closely.

7.2 Circadian Misalignment

A misalignment within the circadian rhythm can occur due to changes of the external environment relative to the internal rhythmicity, as it appears in jet lag disorder or shift workers. Such changes can lead to a shortened sleep length that has been shown to be associated with an increased risk of other diseases like obesity, diabetes, hypertension, and a reduced immune function (Cohen et al. 2009). On the other hand, changes of the endogenous circadian system itself can lead to changes in the sleep–wake cycle, as it appears in patients with delayed sleep phase disorder (DSPD) or advanced sleep phase disorder (ASPD) (Sack et al. 2007).

7.2.1 Delayed Sleep Phase Disorder

DSPD patients have trouble falling asleep and complain about sleepiness in the morning. They will seek advice to readjust their rhythm because of social and occupational demands (Regestein and Monk 1995). These patients may worsen their situation as they may administer self-medication with hypnotics or alcohol. The status may lead to a conditioned insomnia. DSPD is estimated to account for up to 10% of chronic insomnia patients. Reports on epidemiological data is limited, one study suggests a prevalence of 0.17% in the general population (Schrader et al.

1993), others report higher prevalence in young adults and adolescents (7–14%) (Regestein and Monk 1995). Stress and occupational requirements that continue into the late evening may perpetuate the delayed sleep phase. Research has shown that patients with DSPD have a hypersensitive suppression of melatonin by light (Aoki et al. 2001); in addition, lack of appropriate light in the morning may exacerbate the condition, as patients tend to sleep later due to the phase shift (Ozaki et al. 1996). Bright light therapy is one of the most commonly used treatments for DSPD (Morgenthaler et al. 2007) as exposure to bright light in the morning for 1–2 h resets the human circadian clock by advancing the phase of the circadian rhythms (Czeisler et al. 1989). Studies have shown that bright light exposure was able to sufficiently suppress melatonin secretion and reset phase effects on the human circadian system (Lewy et al. 1987). While high-intensity bright light (3,000–10,000 lux) induces the greatest impact on the circadian system, even modest intensities (50–600 lux) can produce notable phase shifts, if presented to individuals, who have been living in dim-lit environments. Three cycles of exposure to as little as 12 lux for 6.5 h produced phase shifts (Duffy and Wright 2005). And finally, intermittent exposure to bright and dim light has been reported to produce almost as much phase shifting as a continuous exposure (Gronfier et al. 2004) – which in turn means that light of lower intensity and even intermittently presented could alter the circadian phase and could therefore be regarded as a stressor to circadian rhythm if exposed to at an undesired time.

The regime of bright light therapy is especially effective if patients avoid bright light in the evening (Rosenthal et al. 1990). The administration of the sleep-inducing hormone Melatonin in the evenings has demonstrated to effectively reset the circadian clock (Kamei et al. 2000), but due to the limited number of clinical studies a standardized approach has not yet been developed.

7.2.2 Advanced Sleep Phase Disorder

Patients with advanced sleep phase disorder wake up earlier relative to the external environment and experience feelings of sleepiness in the evening (Reid and Zee 2009). Phase advancement in ASPD is relatively rare in younger adults as it seems to be associated with aging (Jones et al. 1999); a prevalence of 1% has been estimated in middle-aged adults. Hereditary correlation has been shown in several family cases (Jones et al. 1999) but the general pathophysiology is yet unclear, although a shortened endogenous period in these patients of less than 24 h has been suggested (Jones et al. 1999). Treatment of ASPD has shown to be effective with bright light therapy in the evening as it improved sleep efficiency and delayed the phase of the endogenous circadian cycle (Lack 1993), some patients however had difficulties complying with the regimen (Campbell 1999). Little data exist about the effectiveness of melatonin as a treatment of ASPD and it is unclear if possible sedative effects of melatonin might interfere with daytime function (Reid and Zee 2009).

7.2.3 Free Running Disorder

Free running disorder (FRD) is a condition in which patients exhibit a steady drift of 1–2 h of their sleep–wake period each day. If these patients try to adhere to the conventional sleep–wake times, they often complain about insomnia, early morning awakening, and daytime sleepiness (ICSD-2 2005). FRD is commonly reported in blind people who lack entrainment through sunlight zeitgeber (Eliott et al. 1971); an estimated 50% of blind individuals have FRD. FRD seems to be rare in sighted people (Weber et al. 1980), some cases reported FRD in patients following a head injury. FRD patients show positive results to non-photic entrainment such as social and work schedules; also some blind individuals seem to respond to bright light therapy despite their lack of visual perception. It seems conceivable that these patients suffer from a blindness that does not affect the ganglionic photoreceptors recently associated with photic input as a zeitgeber. In addition, melatonin, administered 1 h before bedtime, seems to be effective in treating FRD in blind individuals (Sack et al. 2000).

7.2.4 Shift Work Disorder

Shift work at night can have a significant impact on the circadian rhythm as it misaligns the individuals' activity from social and physical zeitgeber and hinders the individual to participate in a normal schedule of daily activities. Shift work–sleep disorder (SWSD) typically presents itself as insomnia and daytime sleepiness, chronic fatigue, although the degree of the symptoms may vary, as some shift workers have less difficulty than others and as factors like age, work schedule, access to leisure activities, and other sleep-affecting illnesses, like sleep apnea or narcolepsy, can influence the ability to cope with SWSD (Folkard et al. 1978).

The prevalence of SWSD is potentially high as about 20% of the workforce in industrialized countries is required to attend to night shift work (Presser 1999). Reports suggest that 1–5% of the general population in western countries and approximately 30% of shift workers suffer from SWSD (Drake et al. 2004). Many shift workers report sleep difficulties after night shifts, after awakening from a non-refreshing sleep and daytime sleepiness that even can persist on the days off after the night shift (Knauth 1981). This may pose a problem for the individuals' activities even though the shift work period has passed. The return to a normal sleep–wake pattern is dependent on the speed and direction of the shift rotation (Reid and Zee 2009).

Gastrointestinal complaints are found more often in shift workers than in day time workers, which are also attributed to changed eating habits mostly dictated by work schedules (Waterhouse et al. 2003). Such complaints include alterations of appetite, constipation, dyspepsia, and abdominal pains. Twenty to seventy-five percentage of shift workers compared to 10–25% of day time workers report such complaints, gastric ulcers occur more than twice as often (Segawa et al. 1987). It has

been shown that people working in night shifts exhibit a significantly higher risk of developing cancer (Viswanathan and Schernhammer 2008).

With regard to metabolic changes, higher plasma concentrations of LDL (>3.3 mmol/L), triglycerides (>1.7 mmol/L) and a lower HDL plasma concentration (<1.0 mmol/L) in a shift working population (Karlsson et al. 2001) have been reported. Also it was found that night shifts are associated with decreased glucose tolerance and increased insulin resistance and lipid intolerance (Lund et al. 2001). A report found that 2 h postprandial breakfast plasma glucose levels in eight shift workers increased from 99.9 ± 4.5 mg/dL when aligned to the circadian cycle to 132 ± 13 mg/dL when misaligned. The glucose level increase occurred despite a concomitant increase of insulin (23.3 ± 5.6–49.9 ± 14.0 μIU/mL), which suggests that the insulin sensitivity had dropped while misaligned. Also an abnormally high level of plasma cortisol, previously mentioned as playing an important role during stress and the regulation of the circadian rhythm, was found in misaligned shift workers at the end of the waking and beginning of the sleeping period, which could further contribute to insulin resistance and hyperglycemia (Dinneen et al. 1993).

As mentioned, the possibility of leisure and physical activity of shift workers is limited, as these activities are generally scheduled to a diurnal rhythm and there are little arrangements to meet the demands of the shift worker (Baker et al. 2003). Many shift workers wish to but cannot engage in desired leisure activities as day-workers; therefore, shift workers have problems maintaining their physical fitness compared to day time workers. This circumstance adds up to the already mentioned changes of increased plasma lipids and decreased glucose intolerance.

It also has been shown that physical activity increases the duration and the quality of nocturnal sleep and that physical activity may be beneficial to the shift worker in improving sleep quality as well as influences their fatigue levels and mediates favorable changes in physiologic functions (Youngstedt 2005). It was found that the amount of slow wave sleep (SWS) is important for brain restoration and that SWS is increased by physical activity (Youngstedt 2005). Thus, the combination of a declined sleeping pattern through shift work in combination with the lack of an otherwise brain restaurative SWS due to less physical activity even worsens the situation.

Recently, abnormalities of neuropeptides such as ghrelin, leptin, and orexin have been associated with sleep restriction, which may in turn affect appetite, the risk of overeating, and eventually obesity (Atkinson and Davenne 2007). Leptin, for example, was found to be 17% lower in misaligned shift worker over the entire misaligned period compared to when aligned normally ($p < 0.001$) (Scheer et al. 2009). These substances may also affect non-exercise thermogenesis as a variable component of energy expenditure which was shown to account for different amounts of weight gain in rats. However, little is known about the influences and relationships between sleep, metabolism, body mass changes, and thermoregulation – and therefore, to close the circle, the circadian rhythm.

Treatments for SWSD consist of improving sleep quality through an optimal sleep environment (quiet and dark) as well as administration of melatonin and bright

light therapy in order to realign the circadian rhythm (Morgenthaler et al. 2007) which have to be shown successful to match sleep propensity with the desired sleep time (Sharkey et al. 2001). In bright light therapy, various intensities, ranging from 1,200 to 10,000 lux (Smith and Eastman 2008), and different exposure times, both continuous and intermittent (Eastman et al. 1995), have shown to be successful. Wearing shaded eyepieces to avoid sun light exposure at an undesired time can help improve phase adjustment even without bright light therapy. Treatment should also encompass the social environment of the individual and therapy plans need to be individualized (Reid and Zee 2009).

7.2.5 Jet Lag Disorder

Jet lag disorder (JLD) is a misalignment of the external zeitgeber and the internal circadian rhythm due to long distance travelling across time zones. The arising symptoms may vary and are usually temporary and depend on the number of time zones travelled and the direction of travelling. Eastward travel usually results in trouble falling asleep while westward travel brings difficulty staying awake (Boulos et al. 1995); other accompanying symptoms are general malaise, gastrointestinal illness, and impaired performance (ICSD-2 2005). JLD, due to the long journeys that often take place in relatively small compartments (economy class), is often accompanied by a nonspecific travel fatigue.

Several reports suggest that older individuals may be less prone to JLD and its symptoms (Tresguerres et al. 2001). In contrast, another report showed that middle-aged groups (ages 37–52) had more fragmented sleep and felt less alert when compared to younger individuals (ages 18–25) during a 6 h time advance (Moline et al. 1992).

Treatment usually aims at adjusting the internal circadian system to the new external setting. Therefore behavioral modifications like seeking social and occupational activity according to the present time zone, as well as likewise exposition and avoidance of light can help to diminish the jet lag symptoms (Waterhouse et al. 1997). A general recommendation for travel up to eight time zones would be to seek bright light exposure in the morning after eastward travel and in the evening after westward travel. As in SWSD, the wearing of shaded goggles may help to avoid light exposure at undesired times (Smith et al. 2008). If the time zones crossed exceed 8–10 during eastward travel, it might be more sensible to treat developing jet lag symptoms like a westward travel, since advancing the internal clock is commonly more difficult than delaying it (Takahashi et al. 2002). Entrainment to the new local time can be accelerated using bright light therapy and/or the administration of melatonin (Boulos et al. 1995). If possible, travelers might consider to gradually adjust their sleep–wake cycle to the time zone of the destination prior to departure. Individual adjustments might be necessary if the traveler had prior jet lag symptoms from previous journeys or had other atypical circadian orientations like from shift work.

7.3 Sleep and Circadian Rhythm Under Extreme Environments Related to Human Space Flight and Exploration Missions

Understanding the conditions of how the circadian clock is paced and also shifted which has been investigated under normal conditions of working life (see above, e.g., shift work disorders and jet lag) provides the basis to investigate and to understand the conditions observed in humans subjected to unusual, extreme and somehow life-threatening extreme environmental stressors. Extreme conditions there can lead to disturbances within the circadian rhythm, and its most obvious reflection, the sleep–wake pattern. Sleep deprivation with its detrimental effects on the organism might occur (Rechtschaffen et al. 2002). Human curiosity and the search for new territories will lead explorers, scientists, and entrepreneurs into inhospitable regions on earth and in space. To mimic especially the full scale of mission-related stressor of long-duration exploration missions, investigations on earth are indispensable. This ranges from extreme deltas of outer temperatures to variable pressurization of spacecrafts and habitats and degrees of confinement.

7.3.1 Hot and Cold Environments

It is commonly known that sleep can be disturbed during a hot summer night. The number of reports about sleep disturbances is proportional to heat waves during summer periods, as reported when a heat wave hit Europe in August 2003 (Ledrans et al. 2004). Researchers have tried to standardize the settings in which sleep under increased temperature conditions could be studied. Heat exposure prior to sleep, such as from sauna visits or from a hot bath, has shown to enhance slow wave sleep (SWS). The change in body temperature has been attributed to cause the change in SWS. Heat exposure during sleep, with an ambient dry bulb temperature between 31°C and 38°C, has been shown to reduce the duration of both SWS and rapid eye movement (REM) sleep (Kendel and Schmidt-Kessen 1973). Also such an increase of temperature often led to sleep disruptions. It seems that heat exposure can lead to different outcomes depending on whether it is administered before or during sleep. Research to scrutinize differences in acclimatization has shown that tolerated heat load during the day leads to an elevation of SWS, while a non-tolerated heat strain leads to a diachronic SWS impairment. Sleep stability and REM sleep have shown to be more susceptible to synchronic changes, as these parameters react to nighttime heat strain (Buguet et al. 1998).

Research in cold exposure and sleep has led to controversial results. Some subjects (male) had a very disturbed sleep (Haskell et al. 1981) while the temperature was at 21°C, whereas women showed to have only little alteration in their SWS (Sewitch et al. 1986). Another test revealed no alteration in sleeping pattern, but leads to a longer wake time before subjects fell asleep (Palca et al. 1986). Overall, these results suggest that cold exposure during sleep has detrimental effects on

sleep, as it increases restlessness and alters the REM phase. This is also substantiated by results found in non-acclimatized overwinterers in the Arctic, who slept in sleeping bags (insulation factor: 9 clo) and from whom polysomnographic measurements were recorded. Sleeping in the cold caused a cooling until the rectal temperature reached 34.9°C from where it began to rise again due to shivering and body movements. This in turn caused several sleep disruptions and awakenings. REM sleep phases were shorter than in euthermic environments, with the shortening being proportional to the severity of the cold stress (Eichenberger et al. 1993). Interestingly, there was no alteration in sleep pattern and no deprivation of REM phases in acclimatized subjects, who were exposed to nine daily cold baths of 10°C, each lasting 1 h, prior to their departure for the Arctic (Radomski and Boutelier 1982). Acclimatization to cold seems to be protective of sleep disturbances found in unacclimatized subjects.

Data on long-term overwinterers showed a decrease in SWS and REM sleep phases with the oncoming of the polar night and increased again until the summer. These findings are being attributed to the changes in daylight variation; interindividual variances can also be caused by different levels of physical activity as it was seen in one study where the SWS increased, while the winter was mild and the subjects engaged in daily excursions to a nearby penguin rookery (Buguet et al. 1987).

7.3.2 Hypobaric/Hyperbaric Pressure

High altitude is associated with a diminished barometric pressure which results in a lowered arterial partial pressure of oxygen. Without additional oxygen, acute mountain sickness (AMS) can occur, depending on the rate of ascent, acclimatization of the subject, and his or her physical exertion. AMS is associated with drowsiness, headaches, a decrease in cognitive and psychomotic functions, and even pulmonary and cerebral edema, as the severity intensifies. During sleep, arterial oxygen saturation decreases even further, thus paving the way for AMS to develop. Also central sleep apnea at high altitudes leads to sleep interruption and it was shown that the sleep interruptions occurring in men due to moderate obstructive sleep apnea were replaced and aggravated by central sleep apnea (Burgess et al. 2006). Insomnia and sleep interruptions seem to be proportional to the altitude with the sleep alteration usually appearing at altitudes above 2,000 m and especially during the first 3 weeks of acclimatization (American Academy of Sleep Medicine AASM 2005). The sleep disturbances varied from decreases in sleep time, awakenings to decrease of SWS (Nicholson et al. 1988); however, a great range of susceptibility exists (Mizuno et al. 2005) and some subjects seem to be rather impervious to sleep disturbances at high altitudes. Also ethnic differences seem to be a factor as Tibetian natives exhibited a longer undisturbed sleep time at 5,000 m simulated altitude than Han newcomers living in Tibet (Plywaczewski et al. 2003).

Sleep in a hyperbaric environment – such as hyperbaric chamber used in diving medicine – seems to be only mildly affected at shallow depths (30 m), where sleep

is slightly disturbed and shortened with a prolonged sleep latency and feelings of fatigue and these effects remain unaltered until depths around 300 m where SWS decreases (Matsuoka et al. 1986). Below 400 m using heliox (mix of helium and oxygen) or trimix (mix of oxygen, nitrogen and helium), the sleep disturbances aggravate further as SWS and REM continue to decrease and awakenings occur (Rostain et al. 1997). Sleep at hyperbaric pressure is highly disturbed, unaffected by the used gas mixture; sleeping patterns return to normal during recompression.

7.3.3 Confinement

Preparation of space missions, which due to technical restriction, requires that the crewmembers live close to each other for prolonged periods of time. Studies regarding the influences of isolation and confined space were conducted with different crew sizes and composition (POLAREMSI, ANTEMSI, ISEMSI, HYDREMSI, EXEMSI, with "EMSI" standing for "European Manned Space Infrastructure", as well as the studies HUBES (HUman BEhavior Study) and SFINCSS (Simulation of a Flight of International Crew on Space Station)). Living in such confined spaces as they exist in current space ships or the international space station could give rise to problems that emerge from cultural or linguistic disparities, disharmonies in crew composition and cohesion, as well as aggravation of mood and thought disorders. The lack of privacy can play an important role as a social stressor (please see definition of stress above) which may lead to interpersonal conflicts and thus further tenses the situation. Several studies on isolation and confinement have shown that in the isolated condition, experiences of interpersonal conflict like arguing, fighting, and withdrawal increased (Kanas et al. 2010). With regard to the circadian rhythm and sleep, it was found during an isolation study of 7 days that stress under the conditions of isolation and confinement can lead to increased levels of sympathetic activity and increased sleep motor activity, which in turn may lead to disrupted sleep (Kraft et al. 2002). On the other hand, there are also studies on isolation in which the participants experienced no subjective changes in their sleep quality and where no objective changes in their sleep could be found (Tobler and Borbély 1993). It may well be that in such cases, the crew composition was a better match leading to a less stressful environment for the participants and thus less negative effects on their sleep.

7.3.4 Space Flight

Space exploration is still the final frontier of science where researchers encounter conditions very different from the normal environment on earth. Weightlessness, also called microgravity, is the most significant alteration and as mission time grew longer from the very first journeys in space, the issue of sleep in space had to be addressed. Aside from microgravity, lack of the familiar day–night cycle, narrow space, noises from fans and servomotors, uncomfortable postures and

missing proprioceptive feedback, as well as excitement, space motion sickness, and uncomfortable ambient temperatures can lead to a shorter and less qualitative sleep. It has also been shown that sleep in space is shortened significantly to an average of 6 h, with occasional shortenings as low as 4 h (Santy et al. 1988). It is therefore not surprising that hypnotics are the second most used medication onboard a space flight, surpassed only by drugs treating space motion sickness. More often mission times are extended to the other end of the spectrum: Recreational and bedtime are delayed and eventually reduced due to operational demands by mission control (Dijk et al. 2001). This effect ceases during long-term missions, as operational demands and overtime occurs most likely during the first few days and weeks of a mission, so that sleep times return to normal during the course of a long-term mission (Gundel et al. 2001).

The lack of natural zeitgeber and the other changes during a mission in space can lead to a misalignment of the internal circadian rhythm and the work–rest schedule directed by mission control. This misalignment, as in shift workers or as seen in studies on jet lag (please see above), can lead to sleep disturbances, wake time sleepiness and a decrease in well-being and operational performance (Dijk et al. 2001). Increased sleepiness usually coincides with the circadian minimum of the body core temperature – being asleep at that time seems to be important for the restorative function of sleep (Zulley et al. 1981). Chronically restricted sleep time and/or sleep in poor quality can give rise to possible risk in the operation during a mission in space – as it does in all other areas of occupation. It was suggested that two nights of sleep restricted to 6 h or less result in increased response times, more errors in reaction tests, slower performance in arithmetic tests, and impaired working memory functions (Van Dongen et al. 2003). The impairments accumulate if the sleep restriction persists. If the sleep is restricted for a total of 14 nights to less than 6 h, the result on the accumulated decrement in performance is equal to two nights of complete sleep loss (Van Dongen et al. 2003). It has been suggested that the total duration of sleep during a 24 h period is the most important factor to secure the restorative function of sleep, whether it be taken uninterrupted or split into an anchor period and additional smaller periods of naps (Mollicone et al. 2007). After all, to ensure fully functional astronauts and their health, the aforementioned sleep disturbing factors need to be removed or at least relieved, like loud noises, uncomfortable quarters etc. To secure optimal vigilance during critical tasks (e.g., extravehicular activities), such tasks should be planned in accordance with the astronauts' circadian phase and sleep history to determine the optimal time (Van Dongen 2004). ESA and NASA programs are being adjusted to further study this area and to develop countermeasures to meet the needs of the astronauts (Mallis and DeRoshia 2005).

7.4 The "Circadian Homeostasis": Examples of Physiological Functions and Malfunctions

Not only do stressful events influence the human circadian rhythm as previously discussed but also do changes within the circadian cycle alter the ability to respond to stress and physical demands on human subjects.

7.4.1 Physical Performance

A possibility to determine the impact of sleep deprivation on the ability of physical performance is to measure the time to exhaustion (TTE) of human test subjects.

Research has shown that individuals' TTE after sleep deprivation ranged from a 5% improvement to a 4% decrement compared to TTE of non-sleep-deprived individuals (Pickett and Morris 1975), though unknown data about dietary intake and caffeine consumption may limit these findings. Furthermore, sleep deprivation has been shown to reduce the evaporative cooling function as well as dry heat loss in warm environments (Sawka et al. 1984), thus reducing the body's thermoregulatory ability. Another report revealed less running distance covered by eleven male test subjects after one night of sleep deprivation compared to the same group in a control phase with normal sleep. The test subjects, despite running less distance, exhibited similar perceptions of effort (RPE) during both trials, thus indicating that an altered perception of effort (similar perception of lower running speed) accounts for the decrease in performance after sleep deprivation.

7.4.2 Cognitive Function

Sufficient sleep is necessary for memorizing and recalling, in both motoric and cognitive learning. PET scans revealed that during sleep, the same cerebral areas were active as they were while practicing a motoric function (Marquet et al. 2003). Subjects were able to perform a certain motoric function previously practiced about 11% more sufficiently after sleep compared to subjects, who had to stay awake. Mice exhibited a memory decline after a sleep deficit that was accompanied by oxidative stress in the hippocampus (Silva et al. 2004).

It also could be shown that sleep inertia – the impaired cognitive performance immediately upon awakening – is also influenced by the circadian rhythm, as the reduction of cognitive function immediately upon awakening is more than threefold more pronounced when subjects are woken at the biological night than during daytime. This suggests an influence of the circadian rhythm to the demands on humans to cope with psychological stress such as in case of on-call emergency workers who need to be alert immediately after awakening. The results of the two previously mentioned relationships between circadian rhythm and physical and cognitive performance suggest that night work in a misaligned individual can lead to a reduced performance both mentally and physically.

7.4.3 Immune Function and Fibrinolysis

Furthermore, studies have supported the reception that sleep loss makes humans more susceptible to infections. Accordingly it could be shown that sleep improves the immune responses in human vaccination studies such as influenza or hepatitis A (Spiegel et al. 2002). The protective role of sleep was demonstrated when the morbidity and mortality of experimentally infected rabbits were substantially

reduced by a longer sleep duration (Toth et al. 1993). It is of interest that not only pacemaker cells of the circadian regulation system in the hypothalamus, but also rat NK cells, mouse macrophages, and human leukocytes exhibit rhythmic expression of clock genes that are associated with the sleep–wake cycle (Hayashi et al. 2007). It has been suggested that substances released in relationship with the circadian regulation like hormones such as cortisol, melatonin, or growth hormone not only play a role in regulation of the immune function of immune cells, but also that such hormones-as well as influences from the sympathetic nervous system and the body core temperature-synchronize the peripheral circadian clocks of immune cells and thereby harmonize the functional rhythm of immunity at the cellular level. It is further speculated that sleep deprivation will disrupt the synchrony between immune cells, thus leading to a desynchronization of immune functions and eventually a deregulated immune response.

In turn, immune responses can also give feedback to the regulation of the circadian rhythm, most likely through pro-inflammatory cytokines – it could be shown that infections and low-dose lipopolysaccharide administration increases sleep in humans (Bryant et al. 2004). In another study, inhibition of TNF-α by a soluble TNF receptor improved fatigue in patients with rheumatoid arthritis (Pollard et al. 2006). Also, neutralization of TNF-α reduced daytime sleepiness in patients with sleep apnea syndrome. It could be shown that TNF-α suppresses the expression of PAR bZip clock-controlled genes Dbp, Tef, and Hlf as well as the period genes Per 1–3 in fibroblasts in the mice liver; TNF-α also interferes with the clock-controlled gene Dbp in the suprachiasmatic nucleus of mice causing prolonged rest periods (Cavadini et al. 2007). These findings that suggest a high integration of the circadian cycle and immune function are further supported by another report in which a systemic immune response through the in vivo administration of lipopolysaccharides (LPS) resulted in an upregulation of the core clock genes Per 2 and Bmal1 in equine blood cells, while a treatment utilizing non-steroidal anti-inflammatory drugs (NSAID) not only inhibited the inflammatory responses but also the upregulation of clock gene expression. While these responses could only be found exclusively in the in vivo model, it suggests a yet unknown pathway between immune modulators and the regulation of circadian clocks in the periphery, which also proposes the high significance of the circadian rhythm to innate immune reactions (Murphy et al. 2007).

It was recently shown that there also exists a circadian rhythmicity in some components of the haemostatic system; tissue-type plasminogen activator (tPA) and its inhibitor plasminogen activator inhibitor-1 (PAI-1) show a marked circadian variation in plasma (Andreotti and Kluft 1991). This variation seems to be even more pronounced in combination with stress. It was shown that exhaustion and chronic exposure to life stressors such as chronic overtime work are associated with decreased early morning fibrinolysis and increased fibrinogen levels throughout the day, which in turn would promote thrombus formation and increase the risk of coronary infarction (Van Diest et al. 2002).

7.4.4 Cancer

With regard to the relationships discussed so far, diseases closely connected to the immunological function such as cancer could also be affected by alterations of the circadian rhythm. It was shown that the circadian regulation system also controls functions responsible for drug metabolism and detoxification as well as cellular proliferation such as the cell cycle, DNA repair mechanisms, and apoptosis (Okyar and Lévi 2008). In experimental models, chronic jet lag accelerated growth of two transplantable tumors in mice. In that model, the expression rhythms of clock genes in liver and tumor cells were significantly altered (Filipski et al. 2009). It was shown that disruptions of the circadian regulation could interfere with the regulation of oncogenes like c-Myc and p53, leading to a upregulation of c-Myc and a downregulation of p53, thus causing genomic instability, increasing proliferation, and eventually the possible accumulation of mutations favoring carcinogenesis (Fu et al. 2002). Accordingly it could be shown that persons working in night shifts have a significantly greater risk of developing cancer like breast, colon, and prostate cancer and non-Hodgkin lymphoma (Viswanathan and Schernhammer 2008).

Likewise, it is notable that certain carcinogenic toxins might not only induce cancer but could also itself alter the circadian rhythm through cytokine responses. Thus, carcinogenic effects of such toxins and changes in the circadian cycle could potentiate themselves as it appears in chronic alcohol abuse or hepatotropic viruses, as they both produce preneoplastic liver lesions and disrupt the circadian cycle in experimental model and in humans (Spanagel et al. 2005). In line with these findings it could be advised that treatment strategies should aim at the prevention or correction of circadian disruption to prevent cancer development and control its progress respectively (Filipski et al. 2009).

In line with these findings, stress, because of its influence on the immune system via hormones like cortisol and through changes of the circadian rhythm on the systemic and peripheral cellular level, should be prevented in order to maintain health and well-being, especially under conditions of space flight, when stressor may act in an additive way.

7.5 Summary

As it was shown in this chapter, the circadian rhythm and its regulation are on the one hand closely connected to and controlled by external influences, most notably the day–night cycle as well as other zeitgeber and on the other hand the circadian rhythm itself has close connections to and influences various physiological functions of the human body. A disturbance of the regulation of the circadian rhythm (e.g., through jet lag or in the shift worker) therefore not only results in obvious effects like sleeping disorders such as difficulties falling asleep, sleeping through and daytime sleepiness which reduce physical and mental capabilities – but it can

also lead to pathological changes in the metabolism of carbohydrates and lipids or have effects on vegetative regulation and immune function, thus increasing the susceptibility to diseases like the metabolic syndrome, cardiovascular disorders, or various types of cancer. Research in this field will help to understand the influence of extreme environments on circadiane rhythms. Investigations in man living in extreme climates or being exposed to isolated and confined conditions will comprehend related changes observed in space crews. This knowledge will pave appropriate countermeasures to alleviate the impact of these changes – especially during long-term-space missions – on health and performance of astronauts.

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Endocannabinoids, "New-Old" Mediators of Stress Homeostasis

8

Daniela Hauer, Roland Toth, and Gustav Schelling

8.1 Introduction

The starting point of endocannabinoid research was in 1964 with the discovery of terahydrocannabinol, or THC, by Raphael Mechoulam and colleagues in Israel (Mechoulam and Gaoni 1965). More than 20 years later, in 1988, Allan Howlett and his group discovered the cannabinoid receptor in the rat brain (Devane et al. 1988). This receptor was called CB1 and binds THC with high affinity. The CB1 receptors are extremely wide spread in the central nervous system and are present in far higher concentrations than any other receptor. CB1 receptors control the coordination of movement, emotions, memory, pain, reward systems, reproduction, food intake and nausea and vomiting (Pertwee and Ross 2002). A second type of cannabinoid receptors was discovered in 1993. In contrast to the CB1 system, CB2 receptors are found primarily in the immune system, GI tract, liver, spleen, kidney, bones, heart, and peripheral nervous system (Vaughn et al. 2010). When following the well known principle that receptors usually have endogenous ligands that bind to it, Mechoulam's group discovered the first endogenous cannabinoid, arachidonylethanolamide or anandamide in 1992. A second endocannabinoid called 2-arachidonylglycerol (2-AG) was revealed by the same investigators in 1995. Cannabinoid receptors, endocannabinoids and the related enzymes are essential components of what is now called the endocannabinoid system (ECS).

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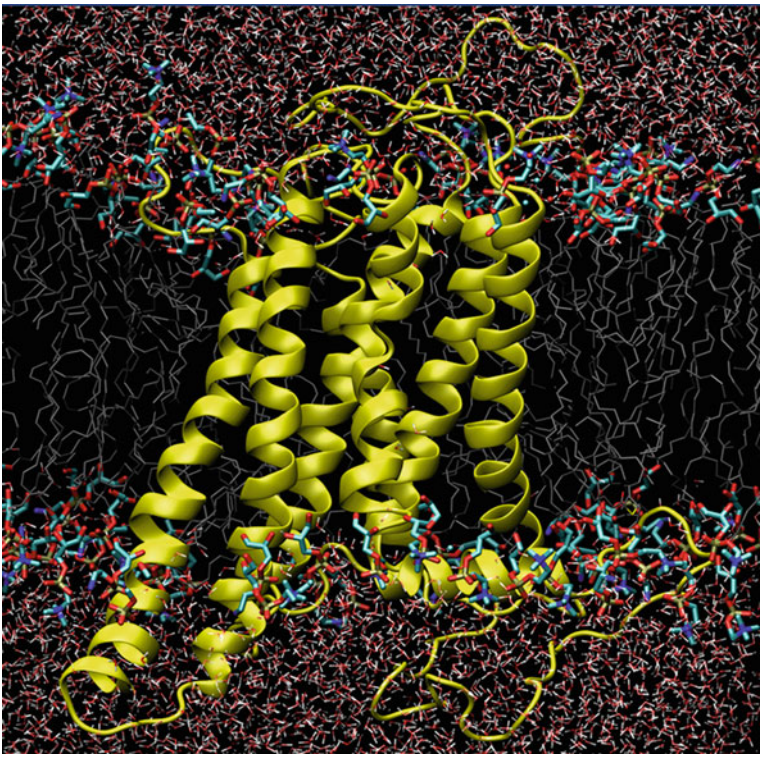

Fig. 8.1 Schematic drawing of a CB1–receptor. The receptor consists of seven transmembrane helices shown in *yellow* on the image (Courtesy of Patricia H. Reggio, Marie Foscue Rourk Professor, Department of Chemistry and Biochemistry, University of North Carolina, with permission)

8.2 Cannabinoid Receptors and Ligands

8.2.1 Endocannabinoid Receptors

The cannabinoid receptors CB1 and CB2 belong to the superfamily of G protein–coupled receptors, coupling to inhibitory G proteins (Gi/o) and inhibit adenylyl cyclase and activate MAP kinase (Jyotaki et al. 2010) (Fig. 8.1). Furthermore, CB1 receptors inhibit presynaptic N- and P/Q-type calcium channels and activate inwardly rectifying potassium channels. Other possible signalling mechanisms involve focal adhesion kinase, phosphatidylinositol-3-kinase, sphingomyelinase, or nitric oxide synthase (Mechoulam et al. 1997). More recent studies point to the existence of at least one other endocannabinoid receptor which recognizes a number of known endocannabinoid ligands, the GPR55 receptor. This orphan receptor represents a new focus of current cannabinoid drug development (Freemon 1972).

8.2.2 Endogenous Cannabinoids (Endocannabinoids)

Endocannabinoids are lipid signalling molecules that behave differently than circulating hormones. They are not stored but synthesized on demand from membrane phospholipids and released into intracellular space via specific membrane transport systems. From there, endocannabinoids in the brain activate presynaptic cannabinoid receptors leading to an inhibition of neurotransmitter release. Degradation and metabolism takes place after cellular reuptake and is performed by specific hydrolases: anandamide is degradaded by fatty acid amide hydrolase (FAAH) and 2-AG mainly via monoacylglycerollipase (MAGL) (Fig. 8.2) (Herrera-Solis et al. 2010).

Apart from the abovementioned and better investigated endocannabinoids anandamide and 2-AG, a number of newer endocannabinoids have been described which include virodhamide, oleoylethanolamide and palmitoylamide. Not all of them seem to be biologically active under physiologic conditions but it is believed that every endocannabinoid has its own properties and function under certain conditions. Table 8.1 gives an overview on names, chemical structure and biologic function of a number of known endocannabinoids.

8.2.3 Endocannabinoid Measurement

The source of peripherally and centrally measured endocannabinoids is not entirely clear but is thought to be nucleated blood cells and brain microglia and neurons, respectively (Herrera-Solis et al. 2010). As previously mentioned, the endocannabinoids are not stored in vesicles or circulate in the blood streem like hormones but are synthesized on demand and immediately degraded. This makes endocannabinoid measurements in blood and tissues difficult to interprete. In particular, endocannabinoid synthesis in known to be continued ex vivo in stored blood samples (Fig. 8.3) which makes immediate sample centrifugation and freezing mandatory (Schmidt et al. 2006; Vogeser et al. 2006). The standardization of endocannabinoid extraction from blood samples and tissues is still in progress and not standardized throughout all research groups measuring enocannabinoids. Nevertheless, endocannabinoid measurements can be performed successfully and if standardized conditions are strictly kept throughout the whole process from collection and extraction until measurements, a "snap-shot" of this fast acting system can give important information about endocannabinoid signalling in well-defined situations.

We developed a method to measure endocannabinoids which is based on high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Vogeser et al. 2006). The latter method provides linear quantification within a range of 0.1–2 ng/mL for anandamide and 0.5–10 ng/mL for 2-AG, respectively. The lower limit of detection of the method (defined as a signal/noise ration >4:1) is 0.025 ng/mL for anandamide and 0.33 ng/mL for 2-AG. In biological matrices, 2-AG (including its deuterated analog) rapidly isomerizes to 1-AG (Vogeser and

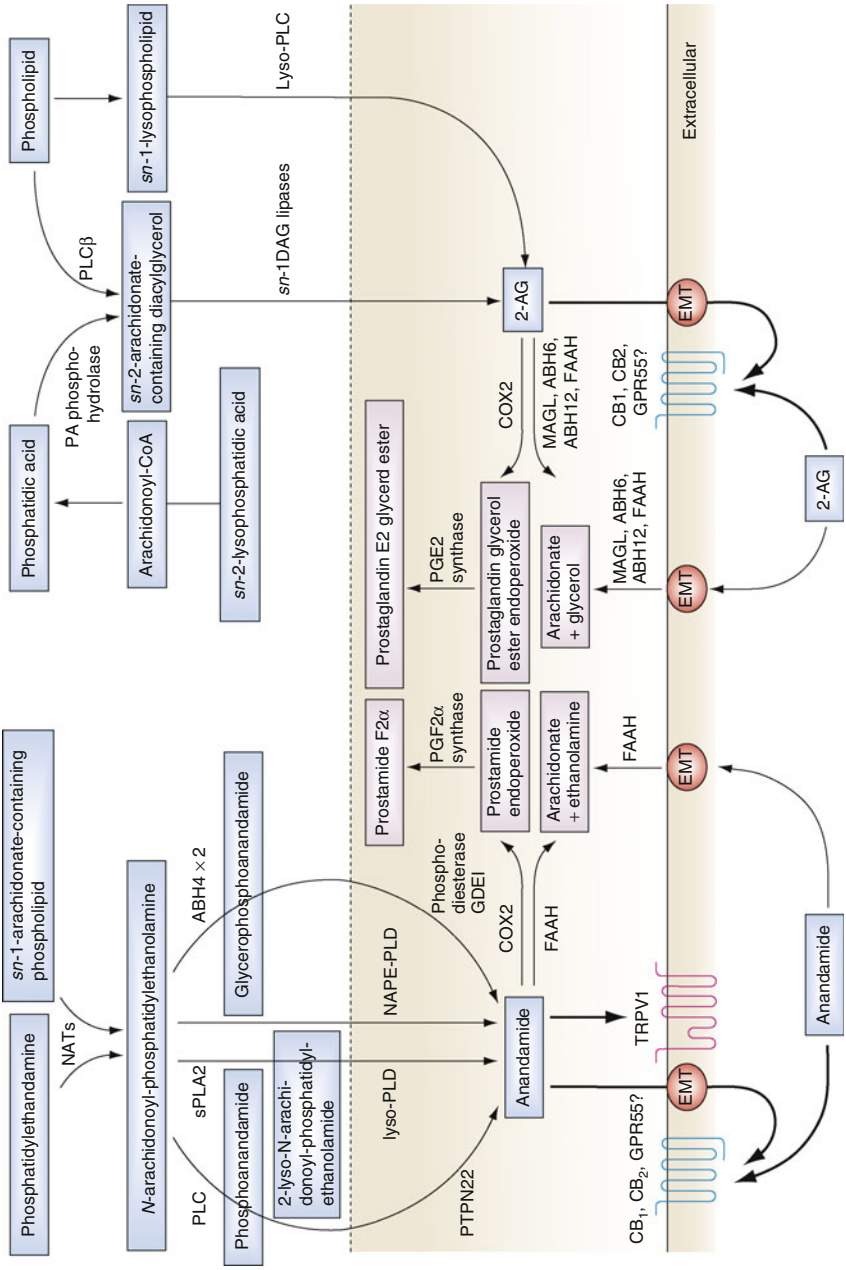
Phosphatidylethandamine
NATs
sn-1-arachidonate-containing phosphatidylethanolamine
N-arachidonoyl-phosphatidylethanolamine
PLC
Phosphoanandamide
sPLA2
2-lyso-N-arachidonoyl-phosphatidylethanolamide
ABH4 × 2
Glycerophosphoanandamide
lyso-PLD
NAPE-PLD
PTPN22
Anandamide
CB1, CB2, GPR55?
TRPV1
EMT
Phospho-diesterase GDEI
COX2
FAAH
Prostamide endoperoxide
Prostamide F2α
PGF2α synthase
Arachidonate + ethanolamine
FAAH
Prostaglandin E2 glycerd ester
PGE2 synthase
Prostaglandin glycerol ester endoperoxide
COX2
Arachidonate + glycerol
MAGL, ABH6, ABH12, FAAH
2-AG
MAGL, ABH6, ABH12, FAAH
CB1, CB2, GPR55?
Extracellular
Phosphatidic acid
PA phospho-hydrolase
Arachidonoyl-CoA
sn-2-lysophosphatidic acid
PLCβ
sn-2-arachidonate-containing diacylglycerol
sn-1DAG lipases
Phospholipid
sn-1-lysophospholipid
Lyso-PLC
2-AG
Anandamide

Fig. 8.2 Pathways of synthesis and degradation of the endocannabinoids anandamide and 2-AG. The biosynthetic pathways for anandamide and 2-AG are shown in *blue*, degradative pathways are shown in *pink*. *Thick arrows* denote movement or action. *ABH4/6/12* αβ-hydrolase 4/6/12, *CB1/2* cannabinoid receptor 1/2, *COX2* cyclooxygenase 2, *DAG* diacylglycerol, *EMT* 'endocannabinoid membrane transporter', *FAAH* fatty acid amide hydrolase, *GDE1* glycerophosphodiester phosphodiesterase 1, *GPR55* G protein-coupled receptor 55, *MAGL* monoacylglycerol lipase, *NAPE-PLD* *N*-acyl-phosphatidylethanolamine-selective phosphodiesterase, *NATs* *N*-acyltransferases, *PA* phosphatidic acid, *(s)PLA1/2* (soluble) phospholipase A1/2, *PLC* phospholipase C, *PLCβ* phospholipase Cβ, *PLD* phospholipase D, *PTPN22* protein tyrosine phosphatase, non-receptor type 22, *TRPV1* transient receptor potential, vanilloid subtype 1 receptor (From Di Marzo (2008), with permission)

Table 8.1 Names, abbreviations and presumed biologic functions of several known endocannabinoids

Chemical name	Abbreviation	Biologic functions
2-Arachidonoyl glycerol	2-AG	1. Heart, circulation system: • May be related to the pathogenesis of Acute Coronary Syndrome (Maeda et al. 2009) • Generated by circulating monocytes and platelets during cardiogenic shock (Maeda et al. 2009) • It was detected in the serum of stable effort angina-patients (Maeda et al. 2009) • Is not produced in the infarct-related coronary artery (Maeda et al. 2009) • Induces relaxations of bovine coronary arteries by extracellular hydrolysis to arachidonic acid and metabolism to eicosanoids (Gauthier et al. 2005) • The concentration of 2-AG responses to orthostatic stress (Schroeder et al. 2009) 2. Behavior: • Stress exposure evokes a significant increase in circulating concentration in women (Hill et al. 2009a) • Basal serum concentrations were significantly reduced in women with major depression (Hill et al. 2009a) 3. Induces full platelet activation and aggregation with a non-CB1/CB2 receptor mediated mechanism (Baldassarri et al. 2008)
2-Arachidonyl glyceryl ether (noladin ether)	2-AG ether	1. Central nervous system: • Neuroprotective by binding to and activation of PPARα (Sun et al. 2007) • Increases the uptake of GABA (Venderova et al. 2005) 2. Induces vascular smooth muscle relaxation in rabbit pulmonary artery (Su and Vo 2007) 3. Attenuates sensory neurotransmission in rat mesenteric arteries (Duncan et al. 2004)

(*continued*)

Table 8.1 (continued)

Chemical name	Abbreviation	Biologic functions
Arachidonoyl ethanolamine (anandamide)	AEA (ANA)	1. Heart, circulation system: • Hypotension (del Carmen García et al. 2003) • Has a dose-dependent coronary vasodilatator effect in isolated rat heart (Wagner et al. 2005) • Causes a PPARγ-mediated, time-dependent vasorelaxation in rat isolated aortae (O'Sullivan and Kendall 2009) • Has a negative inotropic response (Ford et al. 2002) • May be related to the pathogenesis of Acute Coronary Syndrome (Maeda et al. 2009) • Generated by circulating monocytes and platelets during cardiogenic shock (Maeda et al. 2009) • The local level at the culprit lesion of Acute Myocardial Infarction was markedly elevated compared with the systemic level (Maeda et al. 2009) • It was detected in the serum of Stable Effort Angina patients (Maeda et al. 2009) • Has a local production in the infarct-related coronary artery (Maeda et al. 2009) • Increases the duration of the QRS – EKG complex in rats (Krylatov et al. 2007) 2. Basal serum concentrations were significantly reduced in women with major depression (Hill et al. 2009a) 3. Alcohol tolerance may increase accumulation of AEA (Basavarajappa and Hungund 2005) 4. Induces apoptotic body formation and DNA fragmentation in human neuronal and immune cells (Maccarrone et al. 2000) 5. Inflammation: • Inhibits the release of calcitonin gene-related peptide in both skin and spinal cord (Pertwee 2001) • Increases interleukin-6 production by mouse brain cortical astrocytes (Pertwee 2001) 6. Central nervous system: • Increases locomotor activity (Murillo-Rodriguez et al. 1998) • Influences sleeping periods (Murillo-Rodriguez et al. 1998) • Influences working memory (Mallet and Beninger 1996) • Has a role in the neural generation of motivation and pleasure (Mahler et al. 2007)

Table 8.1 (continued)

Chemical name	Abbreviation	Biologic functions
Docosatetraenoyl-ethanolamide	DEA	1. Inhibits the norepinephrine-induced migration of colon carcinoma cells (Joseph et al. 2004)
Dihomo-γ-linolenoyl-ethanolamide (Linoleoyl-ethanolamide)	DLE (LEA)	1. Might be the first natural inhibitor of Fatty Acid Amide Hydrolase (FAAH) (Maccarrone et al. 1998)
N-arachidonoyl-dopamine	NADA	1. Participates in the modulation of T-type voltage-gated calcium channel (Ross et al. 2009) 2. Causes a PPARγ-mediated, time-dependent vasorelaxation in rat isolated aortae (O'Sullivan and Kendall 2009) 3. Induces peripheral blood mononuclear cells-death in end-stage kidney disease patients (Saunders et al. 2009) 4. Is a potent inhibitor of early and late activation events in Ag-stimulated human peripheral T cells (Sancho et al. 2004) 5. Central nervous system: • Has a complex pattern of effects on dorsal root ganglion-neurons and primary afferent fibres (Sagar et al. 2004) • Inhibits both AEA-induced PGE2 and 8-iso-PGF2α in glial cells (Navarrete et al. 2009) • Induces a prolonged presynaptic Ca2-elevation and synaptic activity at sensory synapses (Medvedeva et al. 2008)
N-arachidonyl glycine	NAGly	1. Pain: • Is a newly recognized lipid mediator especially in pain modulation and anti-inflammation (2009) • May provide a novel non-cannabinoid receptor mediated approach to alleviate inflammatory pain (Succar et al. 2007) • Reduced the mechanical allodynia in rat model (Vuong et al. 2008)
O-arachidonoyl-ethanolamide (virodhamine)	O-AEA	1. Potently inhibits the large conductance Ca-activated K-channel (Godlewski et al. 2009) 2. In human bronchial epithelial cells induces an additional Ca2+ entry (Gkoumassi et al. 2009) 3. Provides antagonistic effects against the depressive behaviors in mouse model (Hayase 2007, 2008) 4. Relaxes the human pulmonary artery (Kozlowska et al. 2008) 5. Induces a relaxation of mesenteric arteries (Ho and Hiley 2004; McHugh et al. 2008)
N-oleoyl dopamine	OLDA	1. Is a potent inhibitor of early and late activation events in Ag-stimulated human peripheral T cells (Sancho et al. 2004) 2. Decreases muscle rigidity induced by reserpine in rats (Konieczny et al. 2009) 3. Increases locomotor activity in the rat (Przegalinski et al. 2006) 4. Causes pain sensations via activation of TRPV1 (Bo Tan et al. 2006)

(*continued*)

Table 8.1 (continued)

Chemical name	Abbreviation	Biologic functions
Oleoyl ethanolamide	OEA	1. Has an analgesic effect in a rat model of neuropathic pain (Jhaveri et al. 2006) 2. Exhibited a significant decline during the stress recovery phase (Hill et al. 2009a)
Palmitoyl-ethanolamide	PEA	1. Has anti-inflammatory and anti-nociceptive properties (Jonsson et al. 2001) 2. Exhibited a significant decline during the stress recovery phase (Hill et al. 2009a)
Stearoyl-ethanolamide	SEA	1. Exerts anorexic effects in mice via downregulation of liver enzyme activation (Terrazzino et al. 2004) 2. Has a by nitric oxide regulated pro-apoptotic activity on C6 glioma cells in rat (Maccarrone et al. 2002)

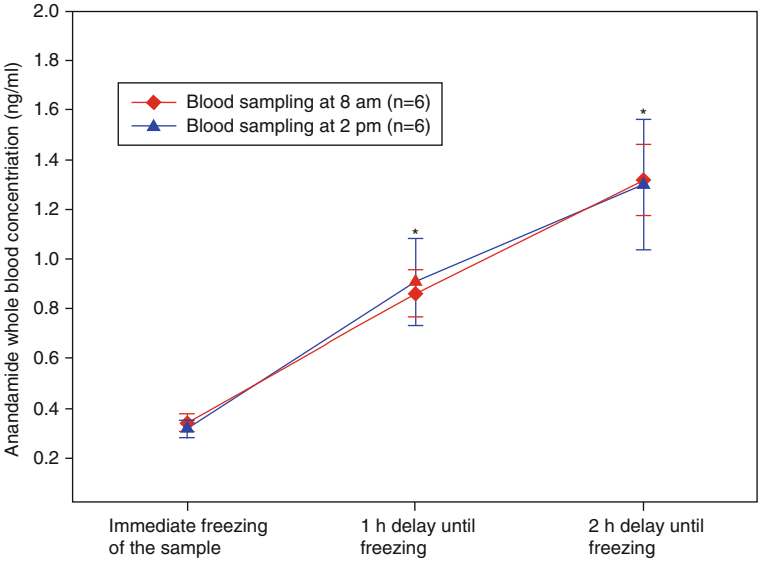

Fig 8.3 Effect of storage of blood samples on whole blood levels of the endocannabinoid anandamide. Samples were either immediately frozen or kept at room temperature for 1 and 2 h. Anandamide levels increased linearly and significantly over time which indicates that blood samples need to be immediately processed (either centrifuged or frozen) to get meaningful results from endocannabinoid measurements. In order to demonstrate a possible circadian influence on anandamide blood levels and this effect, measurements were performed at 8 am (*red lines*) and repeated at 2 pm (*blue lines*) in the same individuals. These measurements did not show any circadian effect although other research groups have reported different results (Vaughn et al. 2010)

Schelling 2007). We and other groups therefore tend to quantify 2-AG as the sum of 1- and 2-esters of arachidonic acid although this question does not appear to have been finally solved (Vogeser and Schelling 2007).

Table 8.2 Effects of endocannabinoids on immune cells

Immune cells	Functions affected	Receptor involved
T-lymphocytes	Proliferation; cell death by apoptosis; Th1/Th2 cytokine secretion, polarization; cell number	CB2
B-lymphocytes	Inhibition of antibody formation; Ig production; Ig isotype switching; proliferation; cell number	CB1 and CB2
Haematopoietic cell line	Cell growth	Non-CB1,Cb2
Macrophages	Decreased inflammatory mediators; antigen presentation; migration; phagocytosis; increased adhesion	CB2
Mast cells	Down modulate mast cell activation; decreased TNF-alpha; decreased mast cell-dependent angiogenesis	Non-CB1,CB2
Dendritic cells	Growth and maturation; apoptosis; recruitment during innate immune response	CB1,CB2
Natural killer cells and neutrophils	Cytolytic activity; chemokines cytokines	Non-CB1,CB2
Cancer cells	Cell cycle arrest; apoptosis; growth inhibitor	CB1,CB2 receptor, TRPV1, lipid rafts

Adapted with permission from Pandey et al. (2009)

8.3 Important Biologic Functions of the Endocannabinoid System to Control Homeostasis in Humans

8.3.1 Immunologic Functions of the Endocannabinoid System

The effects of marijuana smoking or THC on immune cell function were investigated long before cannabinoid receptors and the endocannabinoids were discovered (Klein et al. 2003). The endocannabinoids exhibit complex regulatory effects on the immune system (Pandey et al. 2009). Endocannabinoids are involved in immune regulation by the suppression of cell activation, inhibition of pro-inflammatory cytokine production, nuclear-factor kappa B (NF-kB)-dependent apoptosis and modulation of the functions of T helper subsets (Table 8.2) (Pandey et al. 2009).

The immune effects of cannabinoids and the endocannabinoid system provide promising therapeutic implications in a variety of conditions (Tanasescu and Constantinescu 2010) as cited from the original literature:

- Neurodegenerative diseases:
  - In multiple sclerosis cannabinoid agonists can exert both immunomodulatory and neuroprotective effects (Croxford et al. 2008)

  - In Parkinson's disease, cannabinoid-based compounds might provide protection against the progression of neuronal injury and influence local inflammatory events (Loria et al. 2008)
- Atherosclerosis:
  - CB2 Receptors may influence atherosclerosis by modulating lesional macrophage apoptosis (Freeman-Anderson et al. 2008)
  - Endocannabinoids might also mediate pro-atherosclerotic effects by inducing platelet activation (Mach and Steffens 2008)
- Rheumatic disease:
  - The cannabinoid receptor system may become an important therapeutic target for the treatment of ostheoarthritis and rheumatoid arthritis, because some endocannabinoids have anti-arthritic effects (Malfait et al. 2000)
  - Loss of palmitoylethanolamide (PEA) may contribute to arthritic diseases and CB1 and TRPV1 receptors seem to be important targets in controlling pain from osteoarthritis (Schuelert and McDougall 2008)
- Diabetes and lipid metabolism:
  - In diabetes, cannabinoids may protect against islet destruction by suppressing insulitis and interferon-γ, tumor necrosis factor-α and interleukin-12 mRNA-expression (Li et al. 2001)
  - Rimonabant (a CB1 receptor antagonist) can inhibit adipocyte function and was used in the treatment of obesity. It was, however, withdrawn from the market because of psychiatric side-effects (depression) (van Diepen et al. 2008)
- Allergic asthma:
  - Cannabinoids may be beneficial by ameliorating of cytokine profiles (Croxford and Yamamura 2005)
- Gut and liver disease:
  - Cannabinoids may reduce gut inflammation by direct suppression of proinflammatory mediators (Izzo and Camilleri 2008; O'Sullivan and Kendall 2010)
  - Cannabinoids use of FAAH inhibitors may constitute therapeutic modalities for immune-mediated liver inflammation, hepatic fibrosis, hepatic and intestinal neoplastic disease (Hegde et al. 2008; Izzo and Camilleri 2008)
  - CB2 receptor activation is a promising therapeutic target in gastrointestinal inflammatory states where there is immune activation (Wright et al. 2008)

The effects of exogenous cannabinoids and endocannabinoids on the immune system are broad and involve responses associated with innate, humoral and cell-mediated immunity. The discussed pathways through which cannabinoids act are apoptosis, inhibition of proliferation, suppression of cytokine and chemokine production, and induction of T-regulatory cells (Braun et al. 2011).

There is evidence that cannabinoid effects on the immune system are particularly pronounced under conditions of stress and that they may represent a universally acting protective system against tissue damage of multiple and also non-protein origin (Pacher and Mechoulam 2011).

8.3.2 The Endocannabinoid System and Cardiovascular Functions

8.3.2.1 Vascular Functions

Cannabinoid modulation of vascular tone appears to contribute to the pathophysiology of cardiovascular disorders (Batkai et al. 2004). Endocannabinoids are potent vasodilators that cause hypotension in anesthetized animals and biphasic effects (initial hypertension followed by hypotension) in conscious animals (Ledent et al. 1999). Cannabinoids have also been shown to directly induce vasodilatation in isolated blood vessels (Howlett 2005). Cannabinoid-induced vasodilatation in isolated arteries occurs via endothelium dependent as well as endothelium independent pathways (Su and Vo 2007). Endothelium-derived hyperpolarizing factor released by endothelial cannabinoid CB1 receptor coupled pertussis toxin sensitive G proteins leading to Ca^{2+}-activated K^+ – channel and membrane hyperpolarization of vascular smooth muscle has been proposed as one mechanism (Sunano et al. 1999; Begg et al. 2003).

Some known effects of different endocannabinoids on the vascular system are listed in the following:

- 2-AG ether induces relaxation in vascular smooth muscle, this effect is completely abolished by cannabinoid CB1 receptor antagonists (Su and Vo 2007)
- Anandamide can cause vasorelaxation via 'classical' CB1 receptors, via a putative endothelial cannabinoid receptor, via sensory nerve activation, or the release of nitric oxide, vasoactive prostanoids or endothelium-derived hyperpolarizing factor (EDHF) and decreases blood pressure in mice (Offertaler et al. 2003; Randall et al. 2004). There is also evidence that anandamide interferes with intracellular calcium release in vascular smooth muscle preparations (White and Hiley 1998). Anandamide increased human skin microcirculatory flow and relaxed isolated human pulmonary artery rings in an experimental set-up (Movahed et al. 2005; Kozlowska et al. 2008).
- Virodhamine (O-AEA) causes a full, slowly developing relaxation of the isolated human pulmonary artery, so it is possible that the endothelial cannabinoid receptor in the human pulmonary artery could be a target for the treatment of pulmonary hypertension (Kozlowska et al. 2008).
- Oleandamide (OEA) causes vasorelaxation partly via activation of sensory nerves and partly via an endothelium-dependent mechanism and cyclooxygenase inhibitors increase the potency of OEA as a vasorelaxant (Wheal et al. 2010; Ho et al. 2008).
- N-arachidonyl glycine (NAGly) caused mesenteric arterial relaxation stimulating endothelial release of nitric oxide and through nitric oxide-independent mechanisms, resulting in relaxation of endothelium-denuded vessels (Parmar and Ho 2010).

In addition to modulation of vascular tone, cannabinoids also regulate vascular homeostasis. While cardioprotective properties of cannabinoids have been observed, proapoptotic and anti-angiogenic activities of cannabinoids also have been described

(Zhang et al. 2010; Durst et al. 2007). Anandamide mainly behaves as an inhibitor of angiogenesis (Pisanti et al. 2007); 2-AG interacts with endothelin-1 and may play a role in microvascular function (Chen et al. 2000). An activated endocannabinoid system may modulate vascular repair and angiogenesis, these functions could be important in the maintenance of vascular integrity (Zhang et al.).

In animals, intracisternal application of cannabinoids leads to sympatho-adrenergic activation with increased blood pressure and elevated norepinephrine concentrations (Niederhoffer and Szabo 1999; Pfitzer et al. 2005). Moreover, activation of CB1 in the brain modulates baroreflex regulation (Brozoski et al. 2005). In contrast, peripheral endocannabinoid application lowers blood pressure and decreases norepinephrine concentrations, presumably by inhibiting norepinephrine release from presynaptic neurons (Niederhoffer and Szabo 1999; Pfitzer et al. 2005). Genetic deletion or pharmacological blockade of CB1 receptors has no effect on blood pressure in healthy and normotensive animals, but CB1 receptor blockade increases blood pressure in experimental hypertension, septic shock, and myocardial infarction (Wagner et al. 1997, 2001; Batkai et al. 2004).

8.3.2.2 Cardiac Functions

Administration of anandamide or synthetic cannabinoids causes CB1 receptor mediated hemodynamic changes which are complex, involving phases of both increased and decreased blood pressure as well as changes in heart rate (Randall et al. 2004). Anandamide exerts its cardiovascular effects in mice and rats through cannabinoid CB1 and CB2 receptors and vanilloid TRPV1 receptors (Pacher et al. 2006). A growing body of evidence suggests that endocannabinoid signaling plays a critical role in the pathogenesis of atherogenesis and its clinical manifestations (Mach and Steffens 2008). Increased endocannabinoid signaling is associated with disease progression and an increased risk for acute thrombotic events (Tanasescu and Constantinescu 2010). The presence of CB1 and CB2 receptors in healthy and failing human heart has recently been shown by our group. In particular, on healthy human left ventricular myocardium, mRNA transcripts of CB1 and CB2 receptors were expressed in an almost equal proportion whereas in patients with chronic heart failure, mRNA expression of CB1 receptors was shown to be downregulated 0.7-fold, whereas expression of CB2 receptors was upregulated more than 11-fold. These findings indicate that the endocannabinoid system has a possible role in the regulation of cardiac function under both physiologic and pathologic conditions (Weis et al. 2010).

8.3.3 The Endocannabinoid System and Bone Metabolism

Bone metabolism can be regarded as a constant modelling/remodelling process which continuously renews the mineralized bone matrix. All components of the ECS appear to be present in human skeleton and the ECS has been shown to play an important role in regulating this process (Bab et al. 2008). Bone forming (osteoblasts) as well as well as bone resorbing cells (osteoclasts) are able to synthesise

anandamide and 2-AG and CB1 receptors have been shown to be present in symphatic nerve endings close to osteoblasts. Noradrenergic signals from the central and peripheral nervous system regulate bone metabolism involving beta-2-adrenergic receptors and result in bone loss, an effect which has been demonstrated in stressed patients with depression (Bab and Yirmiya 2010) and put up as a hypothesis in humans during space flight where loss of bone mass is a highly prevalent problem (Strollo 1999). Activation of CB1 receptors in symphatic nerve endings by 2-AG could inhibit nordadrenergic signalling and thus reduce the symphatically driven downregulation of bone formation. A comparable effect of endocannbinoids has also been postulated in patients with the Complex Regional Pain Syndrome, a symphatically driven chronic pain disorder of the extremities associated with bone loss (Kaufmann et al. 2009). The activation of CB2 receptors expresssed by osteoblasts and osteoclasts by stress-activated endocannbinoids could stimulate bone formation and inhibit bone resorption. CB2-receptor deficient knock-out mice exhibit a markedly accelerated bone loss which resembles age-related osteoporosis in humans (Ofek et al. 2006). A single nucleotide polymorphism of the CNR2 gene on human chromosome 1p36 which encodes for the CB2 receptor in women is predictive for low bone mineral density (Karsak et al. 2005). These findings suggest that synthetic CB2 receptor agonists which are available can be administered orally and are free of psychotropic side effects can be used to prevent osteoporosis both on earth (Bab et al. 2008) and maybe even in space during long-term exposure to microgravity.

8.3.4 The ECS, Stress and Control of the HPA Axis

The classic definition of stress as the "body's nonspecific response to a demand placed on it" goes back to the Austrian–Canadian Researcher Hans Seyle who is regarded as the father of modern stress research (Selye 1936). The most important physiologic response to stress is the activation of hypothalamic-pituitary-adrenal (HPA) axis which controls the neuroendocrine response to aversive stimuli. Whereas a fast and timely activation of the HPA–axis is important for adequate functioning and even survival during stress, the rapid shut-down of the glucocorticoid response after termination of the stressful stimuli appears to be equally important. The endocannabinoid system has been shown to play an important role in limitation of HPA–axis activation during and after stress (Steiner and Wotjak 2008).

Recent studies in animals have demonstrated that the systemic administration of glucocorticoids in the absence of a stressor results in a fast increase in anandamide and 2-AG concentrations in limbic structures of the brain important for the regulation of the stress response (Hill and McEwen 2009b). These effects of glucocorticoids are probably mediated by a non-genomic mechanism (Hill and McEwen 2009a). The exposure to acute stress, on the other hand, led to an increase in 2-AG while anandamide concentrations decreased. Pharmacologic or genetic blockade of the CB1 receptor in animals resulted in an exaggregated response to stress which

suggests that stress-induced endocannabinoid signalling in the hypothalamus limits the responsiveness of the HPA–axis to stress.

In order to analyze the relationship between peripheral endocannabinoid signalling and HPA–axis activity in humans exposed to acute stress more closely, we performed a series of parabolic flight experiments (Chouker et al. 2010). In participants with high self-reported stress levels during these experiments (induced by acute motion sickness), anandamide blood concentrations showed an early decrease which was followed by a massive increase in plasma cortisol levels. In contrast, volunteers with low stress exposure who tolerated the experiment well showed an early increase in anandamide and no activation of the HPA–axis. Interestingly, highly stressed volunteers showed almost no increase in plasma 2-AG levels despite a massive activation of the HPA–axis, whereas the opposite pattern was seen in individuals who tolerated the experiment and reported low levels of stress. Changes in anandamide activity clearly preceeded alterations in 2-AG signalling in these experiments. These findings suggest a tonic regulation of HPA–axis responsiveness by anandamide where an early decrease in anandamide activity results in a sensitization of the HPA–axis during the early phase of the stress reaction which is later followed by a strong activation. In contrast, the late increase in 2-AG activity seems to be more related to a limitation of HPA–axis activation which was clearly absent in the participants with a massive stress reaction. The biologic bases for these different patterns of endocannabinoid and glucocorticoid reactivity under stress are currently unknown but recent findings suggest that genetic factors involving glucocorticoid receptor sensitivity may play a role (Hauer et al. 2010). It is of interest to note that the activity of both systems appears to be impaired in chronic stress-related disorders such as depression (Hill et al. 2009b) or post-traumatic stress disorder (Hauer et al. 2010).

8.3.5 Endocannabinoids as Regulators of Stress Response and Sleep

Some of the more intriguing aspects of endocannabinoids is their important role in the regulation of sleep and stress recovery. The endocannabinoid system appears to modulate stress-related endocrine and behavioral responses in order to restore homeostasis after potentially harm- and stressful situations for the organism (Tasker 2004).

These assumptions are corroborated by the observation that highly stressed humans (e.g. war veterans with post-traumatic stress disorder) show a high incidence of chronic marijuana abuse (Sah 2002). Drowsiness or sleepiness are well-known effects in the later stages of intoxication by marijuana (Freemon 1972). Early experiments in mice suggested that anandamide may be a mediator of sleep induction (Mechoulam et al. 1997), and recent experimental evidence has convincingly demonstrated that endocannabinoids are important for sleep regulation with a particularly pronounced effect on rapid-eye-movement (REM) sleep (Herrera-Solis et al. 2010). Sleep depriviation in human volunteers resulted in a significant increase in cerebrospinal fluid concentrations of oleoylethanolamide, an endogenous lipid

messenger that is released after neural injury with neuroprotective and neurotrophic effects (Koethe et al. 2009). In patients with sleep apnea, a disorder characterized by nocturnal sleep deprivation and daytime hypersomnolence, plasma concentrations of oleoylethanolamide were two-fold higher than in healthy controls. These findings suggest that oleoylethanolamide may be part of a neuroprotective mechanism against chronic oxidative stress and promote wakefulness after sleep depriviation (Jumpertz et al. 2010). These observations suggest that the pharmacologic manipulation of endocannabinoid signalling could represent a presumed countermeasure against negative biologic consequences of sleep depriviation during stressful conditions.

8.4 Summary

Recent experiments in animals and humans point to the fact that the endocannbinoid system (ECS) is a critical and highly important regulator of adaptation processes to acute and chronic stress. The ECS affects major stress-sensitive and key organ functions and is involved in the maintainance of immune- and cardiovascular functions, as well as in the pathology of stress associated motion sickness. The ECS is implicated in a complex interaction with other stress–response systems including the sympathoadrenergic and the HPA–axis. Other important roles of the ECS include the regulation of bone turnover with possible important consequences for the treatment of osteoporosis, one of the most prevalent disorders in post-menopausal women. Because as all systems described above (immune, bone, sleep etc.) are significantly affected by space flight, understanding and targeting the ECS seems to be of promising relevance for manned space missions. With this regard, the ECS represents a valid and important example of how findings from basic research, preclinical studies in volunteers and clinical investigations in patients can be applied for space research and find their way back to Earth resulting in a better understanding and new treatment options for common disorders in humans.

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Immune System in Space: General Introduction and Observations on Stress-Sensitive Regulations

9

Brian Crucian and Alexander Choukèr

9.1 General Principles of Immune Functioning

9.1.1 From Physical Barriers to Tailored Immune Responses

The immune system is composed of a wide range of distinct cell types found in peripheral organized tissue where primary immune response occurs (e.g., in spleen, tonsils) and in a vast re-circulating pool of cells in the blood and lymph (-nodes) providing the means to deliver the immune-competent cells to sites where they are needed and also to allow a generalized immune response.

This system has been shaped during evolution to protect the organism against potentially pathogenic bacteria and viruses in a rapid and also specific manner. In the human body, the immune system is the largest organ and consists of more than four trillions of cells weighing more than liver and brain altogether. Hence, it is not the mass of an organ anatomist in former times would consider. Existing at the interface between the environment and the host, the *first line* of host defense is of physical and chemical nature and consists of barriers (e.g., skin) and mucus, enzymes, and acidified thin layers of liquid. Together with the *second line* of defense– the so-called innate immune system – pathogenic germs can be eliminated by phagocytes located in the tissues, e.g., in the lung or in the intestine, at those locations that represent potentially vulnerable areas for germs at the border between the environment and the host. Only higher vertebrates like humans developed the mechanism of reacting to invading germs in a specific manner. This response is targeted exactly to the typical pattern of a germ, allowing also the recognition of the microorganism for a further more rapid and more efficient action in the course of a later, second contact. The

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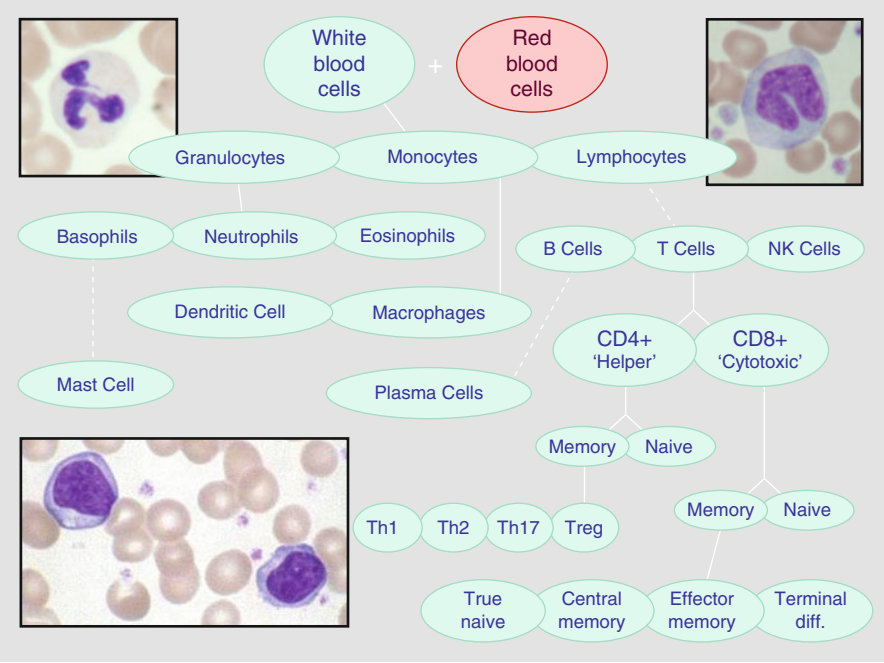

Fig. 9.1 Distribution of primary innate and adaptive immune cell populations in human blood. An analysis of the levels of immune cell subsets can provide information about constitutive immune processes. Populations may increase as cells proliferate during an immune response or contract as cells become sequestered at localized sites of inflammation. Typically, these subsets are quantified by staining specific populations with fluorescent dyes, followed by flow cytometry analysis

immune systems' *third-line* response is based on the action of B- and T-Lymphocytes, and is called the adaptive immunity (Choukèr et al. 2008) (Fig. 9.1).

9.1.2 Innate and Adaptive Immunity

Human immunity is actually comprised of cells of two "distinct" yet interconnected systems, the innate and adaptive immune systems. Innate immunity, mediated by neutrophils, monocytes/macrophages, dendritic cells and NK cells, is the primary line of defense and consists of an immediate (constitutively present) nonspecific response. An innate immune response does not result in immunologic memory; so subsequent responses to the same pathogen are not aided by the previous exposure. In contrast, adaptive immunity is the secondary line of defense. It consists of a delayed response, mediated primarily by lymphocytes, is antigen-specific, and results in immunologic memory. Subsequent re-exposures to the same pathogen result in a significantly greater and more rapid antigen-specific response, due to the presence of circulating memory B and T cells. There are actually two components to adaptive immunity: cell mediated and antibody mediated. Antibody (humoral) immunity is mediated by B cells and antibody production. Antibodies bind to specific antigens, which signal phagocytes to engulf, kill, and remove the target. Antibodies also initiate

target cell killing, via a process termed "antibody-dependant cellular cytotoxicity." In this process, soluble antibodies coat a cellular target which directs cytotoxic lymphocytes to bind and kill that target. The principle of vaccination consists of inoculation of non-virulent pathogen proteins, against which an antibody response is generated. The soluble antibodies and memory cells then persist for years, conferring protection to the host against the specific pathogen for which the vaccine was generated. Cell-mediated immunity (CMI) is mediated by CD8+ cytotoxic T cells, which destroy viral-infected cells, transplant cells, and some tumor cells. Both types of adaptive immunity are regulated by CD4+ helper T cells. The orchestrated action of immune activation, as well as the inverse action of immune suppression to limit inflammation, both processed through endo-para and autocrine pathways, are currently being defined by active research efforts (see Fig. 9.2).

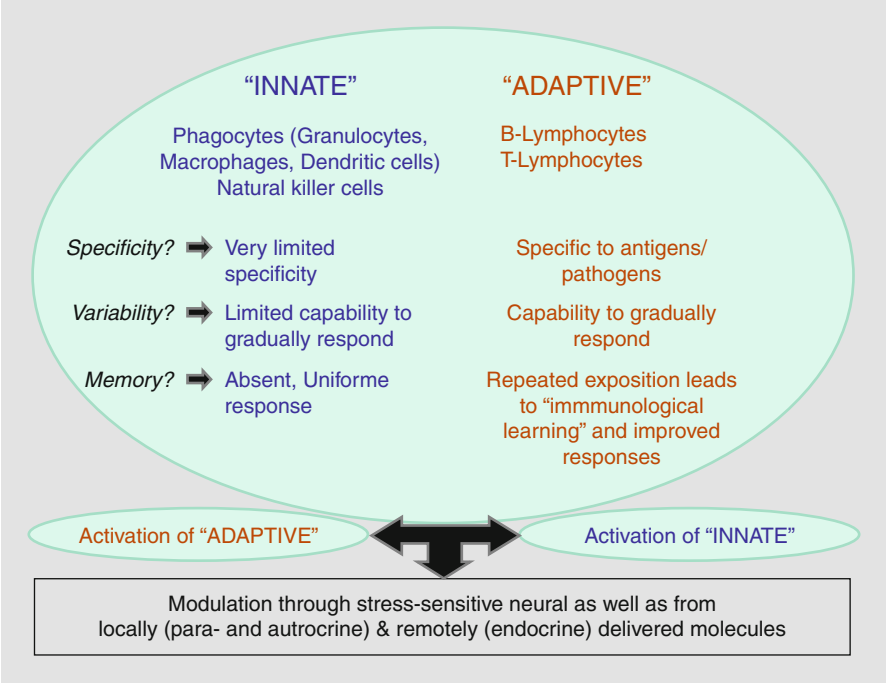

Fig. 9.2 Immunity describes the state of having adequate host defense to protect from infection and disease and is based on a distinct and well-orchestrated interaction of leukocytes (= white blood cells). "innate": Granuolcytes (see Phagocytes) represent up to 80% of all blood circulating phagocytes and one of the prime cell types of the innate Immunity. "adaptive": Leukocytes of the adaptive immune systems consist of antibody-producing B-Lymphocytes and T-Lymphocytes, thereby allowing to *adapt* the degree of the immune responses to the specific pathogens together with the ability to specifically recognize ("learn") the respective antigens in future. The reciprocal activation of the innate and adaptive immune systems guarantees the mounting of a fast, strong, and efficient attack at the first antigen contact and even more, each subsequent time when this pathogen is encountered again. The control – the activation and limitation of responses – is regulated by a very complex and yet not fully understood local (autocrine/paracrine) and remote (endocrine, nerval control) acting messengers (cytokines/hormones) which altogether modulate the sensitivity and responsiveness of the human immune system to protect the human host against thousands of potentially harmful invading pathogens

9.2 The Immune System in Space

Immunological problems of spaceflight were already discovered since the first Apollo missions, where more than half of the astronauts suffered from bacterial or viral infections (Hawkins and Zieglschmid 1975). Also in crew members of Skylab and Soyuz, a reduced reactivity of blood lymphoid cells has been observed (Konstantinova et al. 1973; Kimzey 1977) indicating a potentially alarming observation for long-term space missions. Four decades of investigation clearly indicate that spaceflight affects the human immune system; however, the magnitude and clinical significance of these alterations remains unknown. The knowledge database regarding spaceflight effects on human immunity was recently reviewed (Guéguinou et al. 2009) but the majority of the existing knowledge database consists of post-flight assessments. Post-flight assessments, while providing important baseline information regarding potential in-flight dysregulation, do not necessarily reflect the in-flight status of the immune system. The physiological stress of re-entry and a high-G landing profile, as well as re-adaptation to unit gravity following prolonged deconditioning, both may skew post-flight data. Post-flight findings include alterations in the distribution of the peripheral blood leukocytes, reductions in the function of specific immune cell subpopulations, and altered stress hormone levels.

9.2.1 Peripheral Leukocyte Distribution and Spaceflight

The distribution of the cells of the adaptive (and innate) immune system, usually determined in peripheral blood by flow cytometry, is a measure of in-vivo immune responses. The principle is similar to that of the common "complete blood count" (CBC) measurement; however, the number of specific immune subpopulations identified is much greater. An increased number of subsets are positively identified by staining of cell-specific membrane antigens with fluorescent antibodies, followed by detection and resolution on a flow cytometer. Depending on the specific clinical situation, various subpopulations of immune cells in the peripheral blood (Fig. 9.1) may increase due to clonal expansion, or be reduced as cells migrate out of the blood to localized sites of inflammation or due to a block in maturation processes. Many previous studies have examined the distribution of immune cells following spaceflight, and the data are summarized in Table 9.1. Although specific subset findings may vary among the studies, the most common alterations are increased granulocytes, and reduced lymphocytes and monocytes (lymphocytes mediate adaptive immunity). Among lymphocyte subsets, T cell and NK cell percentages are usually reported to be decreased post spaceflight. Reports on B cell percentages are varied, as are reports regarding CD4+ and CD8+ T cell subsets. In addition to these commonly assessed "bulk" subsets, there are a number of "fine" lymphocyte subsets which have been identified, usually by multicolor flow cytometry (see also Chap. 24). Among these "fine" subsets, Gridley et al. (2009) reported increased CD25+ T cells, Crucian et al. reported increased memory T cells and reduced senescent T

Table 9.1 Representative studies: peripheral leukocyte distribution during or following spaceflight

			Standard cell subsets										
	Subjects	Flight	WBC	Gran	Lym	Mono	T	B	NK	CD4	CD8	Ratio	Other subsets of interest
Taylor et al. (1986)	Human	Short			↓	↓		↓					Humans, post-flight, short duration.
Allebban et al. (1994)	Rat	Short	↓	↑	↓	↓		nc		nc	nc		Rats, post-flight, peripheral blood.
Ichiki et al. (1996)	Rat	Short	↓	↑	↓	↓	↓	↓		↓	↓		
Chapes et al. (1999a)	Rat	Short		↑	↓	↓							
Chapes et al. (1999b)	Rat	Short			↓	↓							
Stowe et al. (1999)	Human	Short		↑	↓	var							Bands increased, integrin-alpha M (CD11b) decreased, L-selectin increased.
Crucian et al. (2000)	Human	Short		↑	nc	nc	↓	nc	nc			↑	Bulk memory naïve variable, CD14/CD16 monocytes decreased.
Mills et al. (2001)	Human	Short	↑	↑		↑		↑	↓	↑			Postflight study; Mission durations of 1 week correlated with increased levels of sympathetic stress hormones and circulating leukocytes; whereas for mission duration of 2 weeks the sympathetic and leukocyte changes were attenuated.

(continued)

Table 9.1 (continued)

			Standard cell subsets										
	Subjects	Flight	WBC	Gran	Lym	Mono	T	B	NK	CD4	CD8	Ratio	Other subsets of interest
Stowe et al. (2003)	Human	Short	↑	↑		var			var	↑			Responses vary between 9 and 16 days missions. 9 days missions = sympathetic responses, 16 days missions = glucocorticoid responses (due to decond).
Pecaut et al. (2003)	Mouse	Short											No change in murine circulating leukocytes. Increase in splenic lymphocyte percentages, decrease in granulocytes. Splenic lymphocytes, reduced T cells, increased B cells, reduced CD4+ and reduced ratio. CD34+ population increased. Data indicates shift in marrow populations, not response to changes in periphery.
Gridley et al. (2003)	Mouse	Short											Short-duration flight: elevated CD25+ lymphs, CD25+ T cells, NK1.1+/CD25+ cells. CD71 decreased during flight.
Rykova et al. (2008)	Human	Short/ Long					nc		↓	nc	nc	nc	
Crucian et al. (2008)	Mouse	Short/ Long		↑	↓	nc	nc	↑	↓	nc	nc	nc	Short duration flight: Elevation in CD4+/CD45RO+ memory T cells, reduction in senescent CD8+ T cells (CD28-/CD244+). Long duration flight: Elevated percentage of CD45RO+ memory T cells for both the CD4+ and CD8+ subsets.
Gridley et al. (2009)	Mouse	Short						↓	↓	↑			Murine spleen: T cell and B cell counts low, NK1.1+ NK cells elevated.
Ortega et al. (2009)	Mouse	Short											Bone marrow: assess maturation/activation of granulocytic lineage cells. No composite phenotypic differences following flight, but were some subpopulation differences. Elevation of CD11b in the R2 subpopulation suggests neutrophil activation. Also, decreased expression of other antigens suggests marrow cells were more differentiated.

cells, and Ortega et al. reported activation and lineage differentiation within murine bone marrow cells (Ortega et al. 2009; Crucian et al. 2008). It is useful to interpret these results, especially findings validated by many studies, and utilize peripheral leukocyte subsets as a monitor of in-vivo immune changes in crewmembers. Granulocytosis, with concurrent decreases in lymphocyte and monocyte percentages, is most likely related to demargination of the neutrophils to the blood in response to the elevated stress hormone levels following landing. It appears that T cell and NK cell percentages are commonly reduced, whereas B cell percentages are commonly elevated. The CD4:CD8 ratio is unaltered or increased, depending on the study. De Rosa et al. (2001) have indicated that changes in "fine" lymphocyte subsets may have clinical significance, even when changes in "bulk" lymphocyte subsets are not evident. Fine lymphocyte and T cell subsets are unique subsets identified by the presence of multiple markers, such as cytotoxic/effector CD8+ T cells, or central memory T cells. Crucian et al. (2008) reported that memory T cells were elevated following spaceflight, whereas senescent T cell percentages were reduced. These changes commonly indicate mobilization of the adaptive immune response associated with spaceflight. It cannot be determined if this mobilization is in response to flight-associated stress, microgravity, the reactivation of latent herpes viruses, or an external infectious agent.

9.2.2 Observations on Immune–Stress Responses in Space

There have been comparatively few in-flight immune studies. Pierson et al. found latent viral reactivation occurs during short duration flight (Mehta et al. 2000a, b; Payne et al. 1999; Pierson et al. 2005; Stowe et al. 2001; Mehta et al. 2004). DTH responses, mediated by monocytic infiltration and memory T cells, have been shown to be blunted during long-duration space flight (Cogoli 1993; Gmünder et al. 1994). Potential causes for this phenomenon include crewmember stress, isolation, disrupted circadian rhythms, microgravity, and radiation as presented in the chapters of Part 3 of this volume. In fact, it is likely that a synergy of these factors is responsible for the observations.

To date, a comprehensive long duration in-flight assessment of immunity, adverse clinical reactions, and physiological stress have to be performed and further comprehensive immune studies are in progress for implementation onboard the International Space Station. To prepare for space flight the knowledge gap on the role of one or more of the distinct stressors in space can be bridged by investigations which address selected stressors – e.g., only μG conditions, or confinement without the effects of low gravity – in appropriate Earth analogue conditions. Analog experiments are of critical importance to simulate and to investigate the effect of environmental factors likely to affect immunity in space as well. These factors include: long-term bed rest in 6° Head-down tilt (to simulate weightlessness) as well as confinement; (Ant-)Arctic overwintering; isolation chambers (MARS500); and underwater habitats (NEEMO, see Chap. 31). Such ground analogs can serve as a suitable earth-bound simulation experiment to mirror some mission-related stressors, but in

a terrestrial location. Although a full comparability of the studies as mentioned below could not have been achieved due to different models, study protocols, and methods, these studies revealed that stress due to confinement induces various changes in neuroendocrine and immune responsiveness.

To date we have learned that stressful situations shown to result in a nerval (e.g., sympathetic) and/or endocrine or auto/paracrine mediated effects on immune responses (Fig. 9.2). This has been exemplarily indicated by a glucocorticoid-mediated immune downregulation which was also associated with reactivation of mostly dormant virus (see Chap. 16). In accordance to these observations, other groups demonstrated dynamics and severity of immune (dys-) function in long-term as well as short-term approaches, respectively. For instance, the consequences of confinement and stress-associated neuroendocrine and immune change have been investigated for different durations during bed rest, e.g., under the conditions of 120 days 6° head-down-tilt (HDT) (Choukèr et al. 2001) or in confinement from 10 days (Shimamiya et al. 2004) to 60 days (Hennig and Netter 1996), to 110 days up to 240 days (Choukèr et al. 2002), and T cell dysfunction became evident within the peripheral blood mononuclear cell compartment, including a paradoxical atypical monocytosis associated with altered production of inflammatory cytokines (Tingate et al. 1997). Moreover, more specific information on changes of the human immune system have been described very recently from a month-long study in the Canadian arctic (Crucian et al. 2007) or in the Concordia base in continental Antarctic environment (Crucian et al. 2011) indicating that several stress responses and specific immune changes were quite similar to those observed in astronauts after space flight. It was found that stressful conditions of psychological or physical nature under such space (-analogous) conditions can modulate innate and adaptive immune responses, respectively. An important role was ascribed to the catecholaminergic- and glucocorticoid-system to affect the host's immune responses together with direct nerval pathways (Tracey 2002), although other not-yet-so-well-investigated stress-response systems along with the endocannabinoid-system are also found to play a role. The latter has been shown to be activated under the conditions of stress (Choukèr et al. 2010) and to modulate a variety of immune cell functions in humans and animals, including T-helper cell development and chemotaxis. It hence appears that the "immunocannabinoid" system is involved in regulating the brain-immune axis (Klein et al. 2003) which exerts an important pathophysiological control of inflammation (D'argenio et al. 2006) (see also Chap. 8). This strong interaction between nerval, neuroendocrine mediators, and the immune system (as been also reviewed by Macho et al. 2001 and Cacioppo et al. 2002) is to be extended by immunotropic metabolic factors to regulate in a way similar to auto and paracrine regulation, the immune cells function under resting and activating status (see Chap. 13).

Some first and confirmative interaction of space flight stress and the consequences on immune functions have been recently published from rodent research in C57BL/6NT mice flown on a 13-day space mission. When a battery of functional, enumerative, and genetic analyses data from space-flown mice were compared to ground control, immune cells' responsiveness was significantly found to be affected. The Phytohemagglutinin (PHA)-induced splenocyte DNA synthesis was highly

reduced, as it was observed for the interleukin-2 (IL-2) production after activation of spleen cells with anti-CD3 monoclonal antibody. In accordance to that Interleukin 10 levels, a cytokine shown to be involved in the limitation of pro-inflammatory processes was elevated. Interestingly, however, interferon-gamma and macrophage inflammatory protein-1-alpha were increased in Flight mice. Moreover, Gridley and coworkers observed further that T-Lymphocytes (CD3+) and B-Lymphocytes (CD19+) numbers were low in the spleens of mice flown on in this mission with higher percentage of NK cells (Gridley et al. 2009). Interestingly, it was shown also that space flight resulted in significant changes of thymic mRNA expression in T cell signaling regulating genes, including: cytotoxic T-lymphocyte antigen-4, IFN-alpha2a (up), and CD44 (down) and the genes that regulate stress and glucocorticoid receptor metabolism (Lebsack et al. 2010). Along the line of these observations indicating dysbalanced immune functional properties under conditions of stress, it was also observed that cancer-related gene expression patterns were significantly modified after being subjected to the stressful spaceflight environment (Gridley et al. 2009).

However, it is yet unknown if these immune system alterations described are transient or if they would persist for the duration of exploration-class deep space missions. The question remains open, if, why, and when and to which degree adaptation processes, in a bidirectional sense also of conditioning and deconditioning, can occur for immune functions as suggested for other organ systems (Nicogossian et al. 1994). With a prolonged immune dysregulation during exploration-class deep space missions, several potential clinical risks to crewmembers, including hypersensitivities, autoimmunity, infectious disease, and malignancies, can endanger mission success.

9.3 Limitations and New Approaches for Immune-Directed Research in Space

Space flight experimental opportunities are infrequent and expensive to conduct and there are many constraints to performing science experiments in space (e.g., hazard aspect, blood handling, up/download mass limitations, and conditioned storage). During spaceflight missions, collection of biological samples (e.g., blood, saliva, urine) is limited due to constraints on crewmember time and lack of resources such as refrigeration and many of the instruments typically used in medical experiments do not function properly in microgravity. Thus, the majority of space flight data comes from pre- and post-flight studies. Although valuable information has been derived from these type studies, there are also limitations that must be acknowledged. These include a several hour delay between landing and bio-specimen collection, the effects of re-entry stress on biological and biochemical measures, and differences in mission duration. Moreover, some discrepancies can be attributed to methodologic differences, adding to the variety of types and extent of stressors encountered during space missions, altogether resulting in the variability of immune responses assessed during and after space flight (Borchers et al. 2002).

In recent years the scientific community has became aware that some of these limitations can be also overcome by evolved methods, extensively tested and stan-

Fig. 9.3 US Astronaut Leland Melvin collects a blood sample from ESA astronaut Hans Schlegel during the STS-122 space shuttle mission. These samples were collected as part of a NASA study to assess immune function, stress, and viral reactivation during spaceflight. Samples were collected near to landing and live whole-blood was returned to Earth for subsequent processing and analysis

dardized hardware, and by the use of safe reagents and "streamlined" procedures that help to gather reliable data. Also, with improved post-flight logistics, it is now possible to return living blood samples from the International Space Station, which allows a functional determination of immune status during flight, without flying significant analytical hardware to space (Fig. 9.3). Moreover, the combination of knowledge and sharing specimen, to address also the combined action of multi-factorial sources of stress conditions during space flight, will help to more comprehensively explore the consequences on the immune system.

9.4 Summary

Because of the nature of the immune system in protecting the host and maintaining health in a changing environment, it seems to be a "natural" and homeodynamic situation that functions of the immune system are affected during the course of space flight. However, long-duration missions in space and especially interplanetary missions require distinct knowledge on how the humans' immune system will cope with these extreme environmental conditions and to which extent an adaptation can occur. Therefore, fundamental and clinical immunology research is critically needed to allow in a multidisciplinary approach a better understanding of how microgravity,

cosmic radiation, and other stressful mission–associated risk factors can influence the adequate functioning of the immune system. These investigations will also help patients on earth that will clearly benefit from advances in research in clinical immunology to better understand immune function in nominal and off-nominal conditions of life.

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Innate Immunity Under Conditions of Space Flight

10

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10.1 Introduction

Although the vertebrate immune system has evolved highly effective and specific adaptive immune responses, the human immune system still fails us on occasions - infections are the leading cause of death globally. On the one hand "our species is immunocompetent", but on the other hand "it is most unlikely that there has ever been a truly immunocompetent individual who was resistant to all pathogens" (Casanova and Abel 2005). The immune system is intimately related in one way or another to almost all pathological states and diseases, and alterations in immunity in space could potentially lead to adverse clinical consequences. Spaceflight associated immune changes is "an area that should be considered more before we undertake prolonged space voyages" (Guéginou et al 2009).

Human spaceflight is an evolutionary unforeseen situation and no process has prepared the human body and mind for life in the absence of gravity, nor indeed in isolation and confinement. The majority of human life sciences research in space has to date focused on bone and muscle loss, cardiovascular de-conditioning, consequences of fluid (re) distribution, and neuro-sensory adaptation processes during both short and long-term missions. However, during the Apollo missions astronauts developed infections during or immediately following space flight (Johnston 1975). In addition, blood samples taken from the NASA Skylab Mission (1973) astronauts upon return to Earth revealed reduced phytohemagglutinin-induced

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lymphocyte blastoid transformation (Johnston 1977). Following these early findings indicating spaceflight-induced immune system alterations, experimental setups were expanded to include the investigation of natural killer (NK) cells (Chap. 11). The discovery by Janeway and Medzhitov that Toll-like receptors (TLR) are crucial and key in the activation of the innate immune system on Earth (Medzhitov et al 1997) stimulated a new-found interest in the study of the innate immune system, its role in adaptive immunity, and its involvement in human disease states. This eventually led to the realization of the importance of studying and understanding the innate immune system under conditions of spaceflight. An international effort to broaden our knowledge in this field is currently under way.

10.2 The Innate Immune System

10.2.1 The Armory of Innate Immunity

The innate immune system includes different myeloid phagocytes (neutrophils, macrophages, dendritic cells (DC)), natural killer (NK) cells and mast cells, as well as humoral components (e.g. complement factors), which together, attack infectious agents in a non-specific but early manner. The characteristic pattern of innate immune activation involves the release of various chemical factors (e.g. prostaglandins, bradykinin) by injured cells, which results in tissue inflammation. Tissue inflammation leads to the activation and recruitment of cells of the innate immune system. In general, the key steps include the interaction of neutrophils with vascular endothelial cells through adhesion molecules such as β2-integrin and selectins, the recruitment of neutrophils to inflamed sites through chemotaxis, and the recognition of microorganisms by phagocytes through pattern recognition receptors e.g. TLR. Several innate immune effector mechanisms then follow, including i) the engulfment and subsequent destruction of microorganisms by phagocytes. The destruction of microorganisms is achieved via a number of toxic chemicals (e.g. reactive oxygen species) and antimicrobial proteins (AMP) and ii) the activation of the complement system which results in opsonization, cytolysis of infected cells, and clearance of neutralized antigen-antibody complexes (Fig. 10.1). The humoral arm of innate immunity in addition includes pattern-recognition molecules (PRM) and neutrophil extracellular traps (NETs). Pattern recognition molecules, such as peptidoglycan recognition protein short (PGRP-S) and Pentraxin 3 (PTX-3), are soluble compounds stored in the granules of neutrophils that act as antibody-like molecules when secreted. Bottazzi and co-workers (Bottazzi et al. 2010) excellently reviewed how PTX-3 is critical for resistance to Aspergillus fumigatus infection through its opsonic properties and activation of the complement cascade. NETs are formed within minutes upon stimulation of phagocytes or hours upon cytolysis of phagocytes at sites of inflammation (Kumar and Sharma 2010), and are networks of extracellular structures that bind and kill microorganisms. NETs consist of nuclear contents such as neutrophil DNA and histones 'decorated' with granules that exert various functions such as microbial recognition

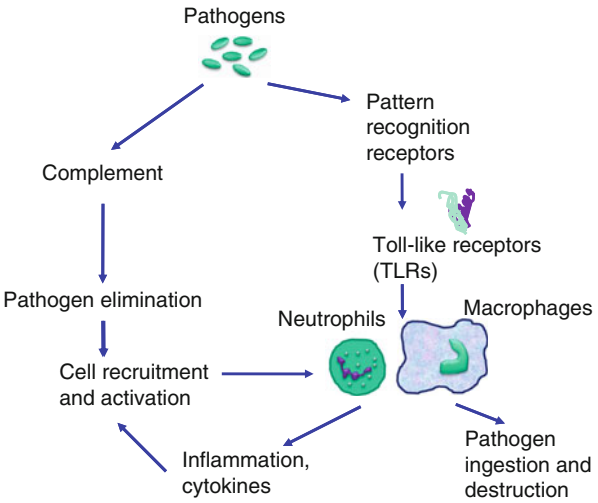

Fig. 10.1 Principles of innate immune responses: The inflammatory responses raised by pathogens include complement activation and the recognition of pathogen structures by so-called pattern recognition receptors. N-formyl peptide receptor 1 (FPR-1), Dectin-1 and Toll-like receptors (TLR) are innate immune-cell bound receptors (either endosomal or surface-associated). TLR recognize molecules widely associated with pathogens (so-called pathogen-associated molecular patterns (*PAMPs*)) that are different from host molecules. Cell to cell interactions, release of chemokines and cytokines promote cell recruitment and, together with complement proteins, pathogen elimination

(e.g. PTX-3, PGRP-S) and antimicrobial activity (Mantovani et al. 2011). NETs have been shown to trap and kill microorganisms such as *Candida albicans* and *Escherichia coli (E. coli)*.

The innate immune system is, in general, not solely involved in combating infectious agents but is however also responsible for eliminating dysfunctional host tissues and cells. Such cytotoxic functions- under certain conditions however- can lead to uncontrolled inflammation and tissue damage (Fig. 10.2). This dual and sometimes conflicting role of innate immunity - to protect the host from infection as well as having the potential to be highly toxic to the host - can be described as a Janus-faced role of innate immunity.

The innate immune system is not a ‘stand-alone’ and separate system within the human immune system but is involved in crosstalk with the adaptive immune system, in particular the activation, recruitment, differentiation, and proliferation of lymphocytes. This process is mediated principally through dendritic cells (DC, Fig. 10.3), which process microbial antigens and present them to naïve lymphocytes, a process known as antigen presentation. This step is considered one of the most important activators of the adaptive immune response (Chaps. 9, 12). In turn, the adaptive immune system has reciprocal actions on cells of the innate immune system, as described below.

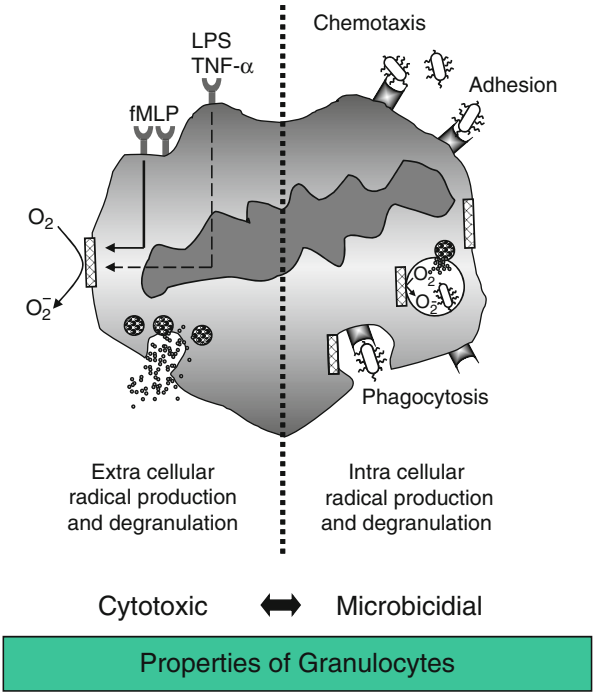

Fig. 10.2 Cytotoxic and microbicidal functions of phagocytes. Lipopolysaccharid (LPS), Tumor necrosis factor alpha (TNF-α), N-formyl methionyl-leucyl-phenylalanine (fMLP)

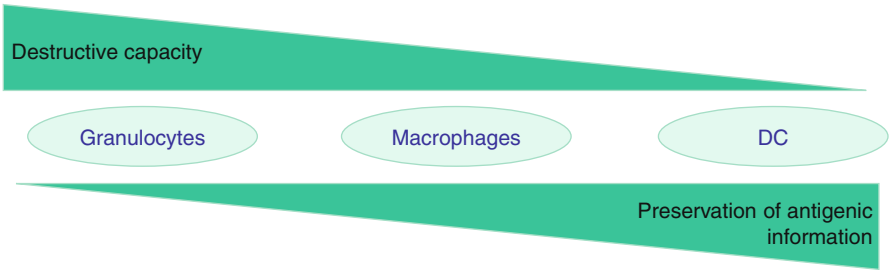

Fig. 10.3 From distruction of pathogens by phagocytes to the initation of adaptive immune responses through antigen presentation. (Modified as seen presented at the Federation of Clinical Immunology Societies FOCIS workshop, Phoenix, Arizona 2010. *DC* Dendritic cells)

10.2.2 The Innate Immune System in Space and Earth-bound Space Analogs

Given the key role the innate immune system plays in 1) the immediate defense against invading pathogens, 2) activation of the adaptive immune system and 3) tissue inflammation, both down-regulation and up-regulation of the innate immune system could have significant adverse effects on the health of astronauts, and potentially disastrous consequence during deep space missions. Therefore two critical questions need to be addressed in preparation for inter-planetary travel are 1) whether the innate immune system of primarily healthy individuals becomes

dysfunctional during the evolutionary unforeseen and highly physically and psychologically stressful conditions of spaceflight and 2) whether the modulation of innate immune cell functions under conditions of spaceflight affects adaptive immunity?

10.2.2.1 Space Missions

Humans are not the only species to have ventured into space. The innate immune system originally evolved in lower order organisms (e.g. fruit fly *drosophila melanogaster*) which serve as useful models for innate immunity research both on Earth and in space. Organisms such as rodents (Allebban et al. 1994, Pecaut et al. 2003, Gridley et al. 2009, Baqai et al. 2010) and amphibians (Bascove et al. 2011) have also been used to investigate certain aspects of immunity during and post spaceflight. The use of animal models allows for better control of experimental conditions and the need for only relatively small laboratory volumes onboard space module. Clearly however, animal models simplify many of the complex and variable physiological and emotional conditions associated with human spaceflight. In particular, stress and associated neuro-endocrine responses in animals, especially in lower order organisms, are unlikely to accurately reflect those that occur in humans. Therefore, this chapter concentrates on studies undertaken in humans alone.

Since 1961 more than 450 (Guéguinou et al. 2009) people have flown in space and all have experienced a myriad of psychological and physical stressors. Of these, only 24 have traveled beyond low-Earth orbit however. During low-Earth orbit our home planet remains always in sight, there is live radio communication with ground control and relatives, the possibility of emergency evacuation, and shielding against solar and cosmic radiation by the Earth's magnetic field. None of these factors will be present during deep-space space missions and the adverse physical and psychological effects when travelling beyond our 'fore-garden', for example to Mars, are likely to be marked.

Neutrophils comprise the majority of circulating leukocytes (50-80%) and are amongst the first cells involved in the immune response to infection. Together with monocytes, they are the main focus of this chapter, whilst the effect of spaceflight on NK cells is reviewed in chapter 11. Analysis of peripheral leukocyte subsets post spaceflight reveals, in general, an increase in neutrophil counts (Chap. 9). However, the vast majority of this data comes from short term (less than 3 weeks) spaceflight experiments (Taylor et al. 1986). Because cell counts do not necessarily correlate with cell functions, Kaur et al. (2004) examined the oxidative burst capacity and the phagocytic abilities of neutrophils drawn from astronauts during missions of either 5 days duration or 9-11 days duration. There were no significant changes during 5 days missions whereas 9-11 days missions resulted in significantly reduced oxidative burst capacity upon activation and reduced ability to phagocytose *E.coli*. Preliminary results from the ongoing ISS-IMMUNO study have also shown reduced neutrophil oxidative burst capacity following stimulation with the synthetic bacterial peptide N-Formylmethionyl-Leucyl-Phenylalanin (fMLP) after 6 months in orbit (Choukèr et al., unpublished). Spontaneous oxidative burst capacity appeared to be augmented however. fMLP is a synthetic peptide that mimics the activity of

bacterially derived peptides and induces the activation and recruitment of neutrophils through their action at specific pattern recognition receptors (e.g. N-formyl peptide receptor 1, FPR-1) (Zhang et al. 2010).

Monocytes form the other major cell types of the innate immune system. One of their functions is to migrate rapidly to sites of inflammation where they then differentiate into macrophages and dendritic cells. Macrophages function primarily as phagocytes, although they can also function as antigen presenting cells and promote the activation, proliferation and differentiation of naïve T-cells. The major function of dendritic cells is to function as antigen presenting cells. In contrast to the consistent changes seen in neutrophil counts, monocyte counts show conflicting results during spaceflight experiments (Chap. 9). As for neutrophils, Kaur (Kaur et al. 2005) demonstrated a reduction in the ability of monocytes to phagocytose E.coli after short duration space flight. In addition, the response of monocytes challenged with the gram negative toxin lipopolysaccharide (LPS) was differentially modulated by spaceflight (Kaur et al. 2008). On the one hand there was a reduction in intracellular pro-inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-1 (IL-1) whilst on the other hand there was an increase in intracellular interleukin-8, a chemoattractant of the CXC-family. Whether this modulation is causally linked to either a modulated expression of CD14 (a co-receptor important in the recognition of LPS) or TLR4 (the major receptor for LPS in human myeloid cells) on monocytes remains to be understood (Guéguinou et al. 2009). As there was downregulation of IL-1, one might speculate that IL-1 dependent activation of adaptive immune responses might therefore also be attenuated.

Whilst the investigation of distinct cellular responses is essential to our understanding of basic immunology, there is a need to understand the clinical consequences of stressors on immunity in an integrated and comprehensive manner, including the crosstalk between the innate and adaptive systems. The delayed type hypersensitivity skin test (DTH, Biomerieux) served as useful tool for monitoring the integrated cellular immune response (Dhabar et al. 1997, 2002). In this test, bacterial and fungal antigens are injected intracutaneously and the degree of skin erythema in response to the antigen challenge measured after 48 hours. Gmünder (Gmünder et al. 1994) observed a reduction in DTH skin reactivity during long term space flight, indicating an overall reduction in cellular immunity. The cutaneous DTH test was phased out in 2002 due to both the risk of sensitization to the antigens and licensing issues. As such, a DTH-like in-vitro test has been developed (Immumed Inc, Germany) and consists of the incubation of whole blood with bacterial, viral and fungal antigens for 48 hours and the subsequent characterisation of the cytokine response. Preliminary data using this assay performed with blood taken from astronauts after 6 month missions appear to strongly corroborate the reports from (Stowe et al. 2001) of significant attenuation of anti-viral immunity as demonstrated by viral reactivation during spaceflight (Chap. 16). Interestingly however, the whole blood immune response following stimulation with e.g. *Candida albicans or Aspergillus niger antigens* was higher post-flight compared to baseline (Fig. 10.4). It remains unclear if this increase in fungal immune responses is due to i) priming of neutrophils and monocytes, to ii) the higher activation of

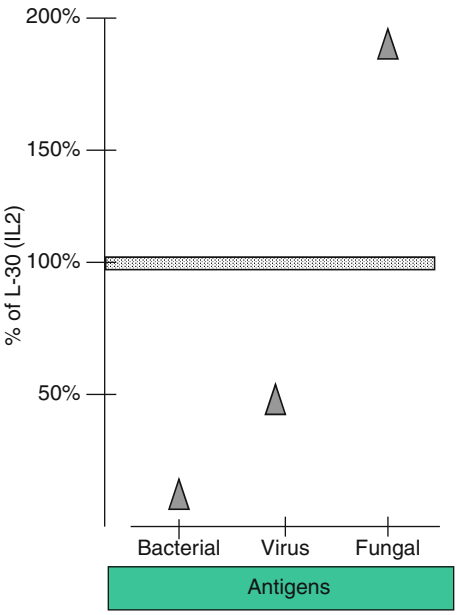

Fig. 10.4 DTH like in vitro test after space flight. Exemplary data shown from one Cosmonaut after return from long-duration mission to the ISS. Whole blood was stimulated at day 7 after return (R + 7) with either bacterial, viral, or fungal antigens; after 48 h incubation at 37°C determination of Interleukin 2 plasma levels was carried out; Data are presented as changes in percentage of the baseline values obtained at one month before launch (L-30); "Bacterial": Tetanus-, Diphteria- and Pertussi- toxoid, "Viral": CMV-EBV-lysate, Influenca protein; "Fungal": Candida- lysate.

phagocytes in response to ß-Glucans in general (a major structural component of fungi), or due to iii) enhanced crosstalk between the adaptive and innate immune systems. The degree and type of microbial, especially fungal, colonization on the ISS (Novikova et al. 2006, see also Chap. 22) might be an additional contributing factor for the observed upregulation of fungal immune responses.

10.2.2.2 Space Analoges

Because of the high logistical challenges and operational costs associated with space missions, earthbound models with characteristics that mimic specific space-flight conditions can serve as useful support research platforms for immune system changes. Space relevant stressors such as microgravity, confinement, and isolation can be addressed individually or in their interactions. Such research platforms have been successfully used throughout the last few decades and allow for greater participant numbers as well as controls. A useful model to simulate conditions of short-lived micro-gravity is *parabolic flight*. A specially equipped airplane performs repeated parabolas resulting in short cycles (about 20 seconds each) of zero gravity followed by hypergravity (1.8 G). The ability of neutrophils to generate reactive oxygen species (ROS) both spontaneously and in response to activation with fMLP +/- TNF-α was studied (Kaufmann et al. 2009). There was an increase in ROS generation by neutrophils in response to fMLP +/- TNF-α after parabolic flight as compared to pre-flight. The spontaneous generation of ROS remained unchanged following parabolic flight however.

The ability of adenosine, an important suppressor of innate immune cytotoxic functions (Sitkovsky et al. 2004), to reduce ROS generation was augmented by

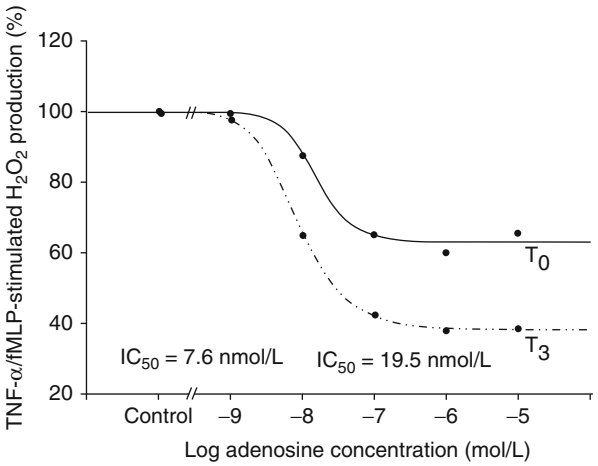

Fig. 10.5 Innate immune responses after gravitational stress (parabolic flight): Fitted dose–response curves for the inhibition of the tumor necrosis factor-α. Tumor-necrosis factor (TNF)-α (10 ng/mL) and *N*-formyl-methionyl-leucyl-phenylalanine (*fMLP;* 10^{-7} mol/L) stimulated H_2O_2 production of polymorphonuclear leukocytes obtainedfrom individuals 24 h before takeoff (T0) and 48 h (T3) after parabolic flight under the influence of increasing concentrations of adenosine (10^{-9}–10^{-5} M). Concentrations of adenosine that inhibit the maximal response by 50% (IC50 value) were calculated by logistic regression analyses of mean values of dose effects determined in each volunteer (Kaufmann et al. 2011)

parabolic flight (Fig. 10.5). This response was modulated by an up-regulation in Adenosine-2A receptor function. This 'STOP' signal on ROS generation might be an evolved mechanism to limit uncontrolled cytotoxic devastation (Kaufmann et al. 2011). The relationship between purinergic pathways and immunity and inflammation has not been explored in space so far but could reveal potential pharmacological approaches to reduce uncontrolled cytotoxic responses by immune cells primed by stress and microgravity. Such a pharmacological approach could be beneficial as a "parachute against uncontrolled hyperinflammation" (Kaufmann et al. 2011).

A further model for gravitational deconditioning in space is 6° head down tilt (HDT) bed rest on Earth, in which the effects of microgravity can be mimicked. Unlike parabolic flight, bed rest allows for the investigation of the long-term effects of simulated microgravity on physiological systems. Head down tilt bed rest results in changes not only of the cardiovascular and musculoskeletal systems, but also the neuroendocrine and hematopoietic systems. 120 days of 6° HDT bed rest led to increased levels of psychic stress and alteration of cortisol circadianicity. HDT bed rest also led to an increase in phagocyte and natural killer cell counts. Up-regulation of the adhesion molecule β2-integrin on the surface of neutrophils and increased levels of interleukin-6 suggested a pro-inflammatory state (Choukèr et al. 2001). β2-integrin allows neutrophils to attach to and roll along the vascular endothelium of inflamed sites and is a key factor in neutrophil host defense functions. Other HDT bed rest studies have confirmed alterations in leukocyte subsets (Stowe et al. 2008),

in addition to increased levels of the pro-inflammatory cytokine TNF-α (Shearer et al. 2009). The effect of bed rest on immunity is controversial however and is likely to be dependent on different durations and conditions of bed rest (Crucian et al. 2009, Mehta et al. 2007).

The lack of gravitational force is only one of many stressors affecting humans in space. *Isolation and confinement* are powerful stressors that can potentially have profound effects on the human psyche. Several studies have been performed in the last few decades testing the effects of variables such as habitat volume, mission duration and crew size and composition during group confinement on stress responses. For instance, the SFINCSS- 99 (Simulation of Flight of International Crew on Space Station) study examined the effects of confinement on 4 healthy men living in a low-volume containment chamber for 110 and 240 days with limited contact with the outside world. The innate immune system showed a trend towards activation as demonstrated by an increase in neutrophil counts together with an up-regulation of β2-integrin on the surface of neutrophils (Choukèr et al. 2002). The use of this analogue facility continued with a study that tested for the psychological and physiological, including immunological, consequences of extended duration of isolation and confinement for 520 days in a simulated Mars mission (MARS500). In 2009, a shorter pilot study lasting 105 days of the Mars simulation flight was completed. Preliminary, unpublished, results from this pilot study indicate that monocyte and lymphocyte counts and pro-inflammatory adhesion molecules on the surface of neutrophils were elevated during isolation. Such up-regulation of pro-inflammatory adhesion molecules is often seen in highly stressed individuals. The simulated MARS500 exploration mission will be completed around the date of the publication of this book. The consequences of such prolonged isolation and confinement on the health of the crew members will be unique and will allow us to judge the risks resulting from a real Mars mission, and develop appropriate mitigation strategies. Over the last decade, Antarctica has also been identified as a suitable platform to investigate the effects of 'real-life' hostile confinement on physiology and is considered a highly useful analogue for lunar and interplanetary missions. In addition, Antarctic bases at high altitude, such as Concordia station at 3200m, serve as useful platforms to test for additional space-relevant stressors such as hypoxia in combination with isolation and confinement (Chaps. 13 and 31).

10.2.2.3 Immune Crosstalk

Cells of the innate immune system, such as macrophages and dendritic cells, contribute to the presentation of antigens to T-cells and subsequent activation and proliferation of T-cells (see Fig. 10.2). Neutrophils are able to modulate this process by either promoting the maturation of DC and monocyte derived DC by cell to cell contact through adhesion molecules, or by inhibiting maturation through the secretion of large vesicles from the neutrophil cell membrane and exposing phosphatidylserine in the outer membrane (so-called ectosomes) (Mantovani et al. 2011; Eken et al. 2010). Neutrophils can also modulate the maturation, activation and survival of NK cells, as well as their cytotoxic and their pro-inflammatory capacity. The latter is predominantly mediated through the production of Interferon-γ and the

direct interaction of neutrophils with NK cells through the intercellular adhesion molecules ICAM-3 and CD18-CD11b. The relationship between neutrophils and NK cells is not unidirectional however, as NK cells are capable of priming neutrophils to produce reactive oxygen species and pro-inflammatory cytokines, resulting overall in an efficient "defensive alliance" (Costantini et al. 2011). The interplay between the adaptive and the innate immune systems can be seen in the efficient co-recruitment of cells of the innate and the adaptive immune system to sites of infection. Distinct T-cell populations (e.g. T-regulatory-cells, T1/17 cells) are capable of modifying the activation status and survival of neutrophils, predominantly through the release of granulocyte monocyte colony stimulating factor (GM-CSF) and TNF (Mantovani et al. 2011). Kaufmann et al. (2009) demonstrated that elevated blood concentrations of GM-CSF and TNF were associated with priming of neutrophils after gravitational stress. Reciprocally, T-cells are guided by activated neutrophils to sites of inflammation by a battery of CC and CXC (Roth et al. 2004). Furthermore, activated neutrophils serve as a major source of mediators that affect B-cell differentiation and functions e.g. through B-cell activation factor (BAFF) (Scapini et al. 2008).

Although modulation of basic phagocyte functions has been shown to occur as a result of spaceflight, further research is needed to evaluate to what degree some of the more complex armory of the innate immune system and its relationships and crosstalk with cells of the adaptive immune system are altered.

10.3 Summary

Endocrine mediated modulation of immune responses is the predominant mechanism of stress-permissive changes in immunity, and occurs primarily through changes in the hypothalamic-pituitary axis and the adrenergic systems (Chap. 6). The brain and the innate and adaptive immune systems when challenged by multiple stressors interact with each other in a multidirectional manner. The intensity and duration of stressor exposure affects these interactions. In general, the modulation of immunity by stress-permissive mediators can be beneficial and support immune responses in the short-term, but, conversely, can become detrimental when theses pathways are continuously challenged (Chap. 3 - allostatic overload) and can result in suppression of cell–mediated immunity. It is clear from the spaceflight immune research data to date, in combination with Earth-based space analogue studies, that the stress of spaceflight adds an extra layer of complexity (Mills et al. 2001, Crucian et al. 2008, Stowe et al. 2011). It remains to be understood why innate and overall cellular immune responses in space do not always follow the same patterns as observed in situations of life stress. Although it is clear that spaceflight alters the innate immune system, our knowledge of innate immune function in space is still limited. The effects of physical stressors such as microgravity and radiation in addition to stress-activated immunotropic hormonal pathways, as well as the presence of an altered microbial environment, need to be understood both separately and in combination. The changes in innate immune responses may also result from a complex crosstalk with adaptive immune responses. To what degree upregulation of

certain aspects of innate immunity can be considered a compensatory mechanism for adaptive immune suppression, and vice versa, in an effort to maintain immunological homeostasis under conditions of stress is unclear and will need a more integrative approach to immunology research in space. Eventually, "understanding the failures of the immune system should make it possible to devise novel ways of making it succeed" (Casanova and Abel, 2005).

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NK Cells Assessments: A Thirty-Year-Old History of Immune Stress Interaction in Space

11

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Natural killer (NK) cells are a form of cytotoxic lymphocytes which constitute a major component of the innate immune system. NK cells were first described in the early 1970s on a functional basis according to their ability to lyse tumor cells in the absence of prior stimulation (Herberman 1974). Today, the NK cell is widely viewed as an efficient non-MHC-restricted effector cell which is capable of patrolling the host and getting involved in the defensive action against infectious agents, tumor cells, and certain immature normal cells. In addition, recent research highlights the fact that NK cells are also regulatory cells engaged in reciprocal interactions with dendritic cells, macrophages, T cells, and B cells. Thus, NK cells may serve as a bridge in an interactive loop between innate and adaptive immunity. Dendritic cells stimulate NK cells which then deliver a co-stimulatory signal to T or B cells allowing for an optimal immune response (Blanca et al. 2001; Vivier et al. 2008). NK cells are able to deliver a response immediately after recognizing specific signals, including stress signals, "danger" signals, or signals from molecules of foreign origin (Fig. 11.1). Suppression of NK activity and/or reduction in the absolute number of NK lymphocytes circulating in peripheral blood has been found to be associated with the development and progression of cancer, with acute and chronic viral infections, chronic fatigue syndrome, psychiatric depression, various immunodeficiency syndromes, and certain autoimmune diseases (Orange and Ballas 2006). In this context, intact natural immunity, as evidenced by normal levels of NK activity and numbers of circulating NK cells, appears to play an important role in health (Vivier et al. 2008). On the contrary, low or absent NK activity is often a sign of disease or an early predictor of susceptibility to disease.

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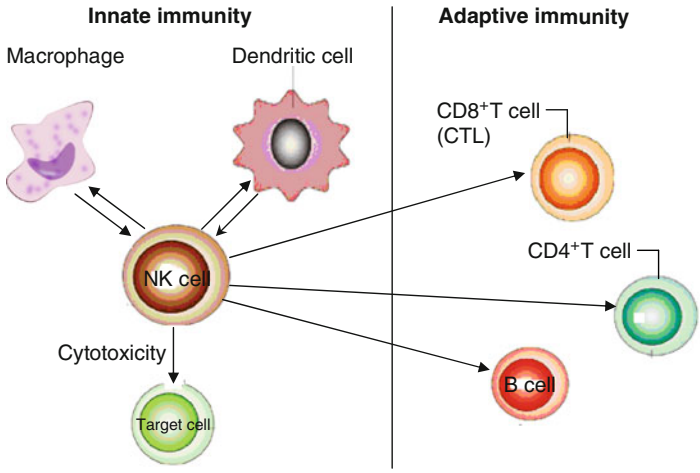

Fig. 11.1 The hypothetical scheme of the potential role of natural killer (*NK*) cells in the immune response: NK cells can kill target cells (transformed or infected cells) and NK cells can regulate innate and adaptive immune responses through a combination of cell surface receptors and cytokines

Because the NK cell appears to be involved in multiple effector, regulatory, and developmental steps of the immune systems' function, investigation of NK cells may serve as an important marker of a clinical risk associated with humans' prolonged exposure to environmental stressful conditions, including stress factors uniquely associated with space travel. The intent of this chapter is to provide a summary of results of the study of the effects of stress factors of extended space flights on the NK cells.

11.1 Natural Killer (NK) Cell Activities Review After Long-Duration Missions: From Salyut to ISS

11.1.1 Cumulative Data

NK cell total cytotoxic activity has been tested on the basis of the amount of non-degraded [^{3}H]RNA that remains in the target K562 cells after contact with NK cells (Rykova et al. 1981). Since the first investigation of the NK functional activity in Russian cosmonaut after 185-day mission on Salyut-6, in 1980, the cytotoxic activities of NK cells were examined after long-duration flights on Salyut-6,7, and ISS in 88 crew members (Konstantinova and Fuchs 1991; Konstantinova et al. 1993; Rykova et al. 2001, 2008). The mission duration varied from 65 to 438 days. These studies showed a high degree of individual variability of NK cell cytotoxic activity. Thus, more than 60% of crew members had reduced NK cell cytotoxic activity from preflight values after long-duration missions (Fig. 11.2). On the first day after landing, the reductions in cytotoxicity were either moderate (decline by 20–50%) in 15

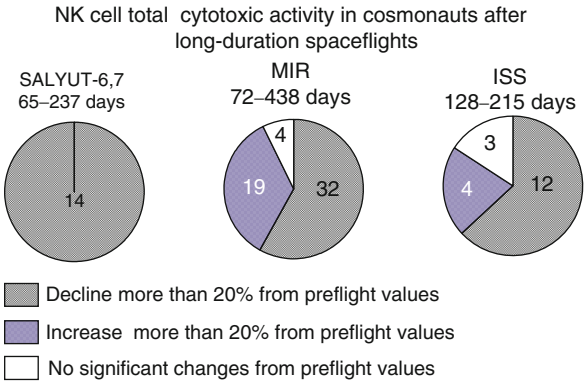

Fig. 11.2 Individual variability of NK cell cytotoxic activity after long-duration space missions (1 day after landing)

of the 74 cosmonauts, in the middle range (decline by 51–90%) in 17 cosmonauts, or in the significant low range (cytotoxicity close to zero) in 7 cosmonauts, respectively. At day 7–9 after landing, NK cell cytotoxic activity of only 4 crew members increased toward their preflight values, but NK cell cytotoxic activity in 16 cosmonauts continued to decrease in their values on day one after landing. Examination of cosmonauts at days 14–19 postflight demonstrated that NK cell cytotoxic activity remained decreased in almost 30% of the cosmonauts.

On the other hand-and in contrast to the Salyut missions of equal duration when 100% of the crew members showed lower NK activities postflight-the first day after the long-duration missions on MIR and ISS, NK cell suppression was not seen in all cosmonauts. In contrast 36% and 21% of the crew members demonstrated even a significant increase in NK cell cytotoxic activity as compared to the preflight values, which then tended back to baseline by the last recovery data point (within 7–14 days after landing).

Analysis of the results obtained from investigation of cosmonauts allowed to identify differential types of NK cell reactions as a consequence of long-duration spaceflight conditions: firstly, a decrease in cytotoxicity on the first day after landing with an increase toward preflight values in 1 week; secondly, the absence of a decrease in cytotoxicity on the first day after landing and a significant decrease in cytotoxicity on the seventh day; thirdly, a decrease in cytotoxicity at days 1–7 postflight; fourthly, an increase in cytotoxicity on the first day after landing with a trend toward preflight values upon examination at later periods.

11.1.2 NK Cells Cytotoxicity Affected by Countermeasures

The assessments of NK cell functions in cosmonauts after long-duration Mir or ISS missions show overall a lower degree of the NK suppression or even an increase of activation which might be not surprisingly when taking into account the countermeasures that have been implemented in the meantime since the Salyut era. The system of countermeasures used by Russian cosmonauts in space flights on board of Mir and ISS included as primary components: physical methods aimed to maintain

the distribution of fluids at levels close to those experienced on Earth; physical exercises and loading suits aimed to load the musculoskeletal and cardiovascular systems; measures that prevent the loss of fluids, mainly, water–salt additives which aid to maintain orthostatic tolerance and endurance to gravitational overloads during the return to Earth; well-balanced diet and medications directed to correct possible negative reactions of the body to weightlessness. Fulfillment of countermeasure protocols in flight was thoroughly enforced (Kozlovskaya and Grigoriev 2004; Kozlovskaya et al. 1995). Recent scientific research has shown that NK cells are highly influenced by physical exercise. The possible important mechanisms behind exercise-induced changes in NK cell function are cytokines, hyperthermia, and stress hormones, including catecholamines, growth hormone, cortisol, and beta-endorphins. Infusion studies mimicking stress hormone levels in blood during exercise indicate that increased plasma-adrenaline accounts for at least part of the exercise-induced modulation of NK cell function (Pedersen and Ullum 1994). Many studies have documented that severe exercise is followed by immunodepression, but moderate regular exercise has a beneficial effect on the NK cells function (Mackinnon 1999) (see also Chap. 27). In this connection, elevated NK cell cytotoxicity after long-duration space missions in some cosmonauts could have been associated not only with the constitutional characteristics of these individuals but also with the performance by the crew members of a set of recommended prophylactic measures, first of all moderate regular physical exercise.

11.1.3 NK Cells, Cytotoxicity Associated with Viral Reactivation?

A further explanation of an increase in NK cell cytotoxicity in some cosmonauts after long-duration space missions is that enhancement of NK cell cytotoxicity was associated with latent viral reactivation during space flight as observed during short-duration missions (Mehta et al. 2000, 2005; Stowe et al. 2001a, b; Pierson et al. 2005; Cohrs et al. 2008). Indeed, a normal NK cell response to a viral infection should be an initial rise in activity, followed by elevated activity during the course of infection, and then a gradual return to baseline activity, while NK cell numbers can be reduced during infectious episodes (Whiteside and Herberman 1989). However, the relationship between the function of NK cells and latent viral reactivation in space flight has not been fully investigated, but the establishment of such a relationship could lead to the development of countermeasures that could prevent any compromises in resistance to infection resulting from exposure to flight conditions.

11.1.4 NK Cell Cytotoxicity Declines Proportionally to Mission Duration?

It seemed of interest to clarify whether the magnitude of the reduction in the cytotoxic activity of NK cells correlates with the length of time an individual remains in the microgravity associated with space flight. The data from all cosmonauts

examined after prolonged missions were divided into four groups based on the length of time an individual remained on board the orbital station: 65–131 days, 145–199 days, 208–241 days and 312–438 days, respectively. No future decline was noted when flight duration increased from a few months to 14 months. Most of the nine cosmonauts, participating in missions lasting 65–131 and 145–199 days two or three times, had similar changes in NK cell cytotoxicity after all flights. In one of these cosmonauts, a decrease in cytotoxic activity was significant after the first spaceflight lasting 65 days aboard the Salyut 7 station, but a reduction was moderate after two missions lasting 152 and 175 days aboard the Mir station. Note that examination of 17 crew members who flew two or three times during 145–199 days demonstrated in some cases a considerable magnitude of changes in NK cell cytotoxicity after the first flight and subsequent missions. Comparison of the degree of changes after repeated exposure to space allows some speculation that some discrepancy could have been associated with some specific features of each mission or differences in the countermeasures that were used, respectively. Therefore it is of importance to note that some crew members, even after mission durations of 12 or 15 months, had rather high NK cell activity – at least during all the measurement periods after landing. These results possibly indicate that the immune system (or some of its components) could become adapted - possibly also as a consequence of countermeasures - to prolonged exposure to space flight conditions.

11.2 Natural Killer (NK) Cell Activities, Subpopulations, and Counts: Results and Considerations from More Recent Long-Duration Missions

NK cells execute their cytotoxicity in a sequential manner, which involves (I) formation of conjugate between NK and target cell, (II) delivery of lethal hit, (III) disassociation of target cell and NK cell. A disassociated NK cell can restart and bind to new targets and finally eliminate them, commonly referred to as recycling capacity or killing frequency (Ullberg and Jondal 1981). In order to define the nature of changes in NK activity after long-duration flights, experiments were performed using single cell-in-agarose assay with mononuclear cells (Grimm and Bonavida 1979). By these assays, it was possible to estimate the capacity of effector cells binding to target cells, the lytic activity of NK cells in conjugates formed, and the percentage of active NK cells. Examination of 27 cosmonauts who lived on-board space stations Mir for 2 to 12 months revealed a significant decrease in the proportion of lymphocytes that bound target cells and the percentage of active NK cells in peripheral blood mononuclear cells within 7 days of landing (Meshkov and Rykova 1995). In most cases, these changes correlated with decreased NK cell total cytotoxic activity in peripheral blood mononuclear cells. However, the percentage of cytotoxic cells in conjugates after the flights did not differ from the data observed before the launch.

The induction of many NK cell effector functions, including cytotoxicity, requires that the NK cell contacts its target cell. Thereupon, a likely explanation of findings, i.e., a decrease in the cytotoxic activity of NK cells in crew members after long-duration

space missions is a defect in the initiation stage in the formation of the lytic NK cell immunological synapse. Because early steps in NK cell synapse formation – adhesion – depend on the integrin family of adhesion molecules (Orange 2008), it is possible that NK cells from cosmonauts do not adhere to their target cells, which results in defective cytotoxicity. This hypothesis seems to be highly probable since after landing, a marked decrease in the cell surface expression of the adhesion molecules CD11b on NK cells as compared to preflight values was observed. In contrast, space flight did not induce significant changes in events that occur following the interaction between a cytolytic cell and its target cell resulting in the directed secretion of lytic granules onto a target cell. At the same time, after 179-day mission on board MIR station in one cosmonaut NK cell total cytotoxic activity was significantly decreased, but the capacity of lymphocytes to bind target cells was relatively unchanged. In this case, the inhibition of the recycling function NK cells, which have already participated in serial killing, may form the the basis of the effect.

NK cells are a heterogeneous subset of large granular lymphocytes. Recent studies have identified at least 48 distinct NK cell subsets, whose significance and function largely remain uncertain (Jonges et al. 2001). A few NK cell subsets, however, have been well defined. NK cells do not express T cell receptors (CD3) on their surface but express antigens CD16 and CD56. There has been debate over the past several years as to whether changes in cellular distribution reflect changes in NK cell total cytotoxic activity. It appears that changes in circulating NK cell number account for most of the change in total NK cell cytotoxic activity after long-duration flights. Thus, in most crew members, a reduction in total NK cell cytotoxic activity after landing was accompanied by a similar reduction in the percentage of circulating $CD3^{-}CD56^{+}CD16^{+}$ NK cells. However, not all effects of long-duration space missions on total NK cell cytotoxic activity can be attributed solely to changes in NK cell number. In particular, two variants of changes of NK cell number were observed. Firstly, the percentage of $CD3^{-}CD56^{+}CD16^{+}$ NK cells was unchanged or even elevated (as compared to preflight baseline values), but total NK cell cytotoxic activity was reduced. Secondly, the percentage of $CD3^{-}CD56^{+}CD16^{+}$ NK cells was significantly decreased, but total NK cell cytotoxic activity was significantly increased. These results showed that investigations of the effects of spaceflight factors on NK cells should include examination of NK cell activity and NK cell counts.

In the recent years, NK cells have been categorized into major groups based on the level of CD56 expression (CD56 bright and dim) (Cooper et al. 2001). These $CD56^{bright}$ and $CD56^{dim}$ subsets are thought to differ in their tissue homing properties due to differential patterns of expression of chemokine receptors and adhesion molecules. $CD56^{dim}$ NK cells comprise the majority of peripheral blood NK cells (approximately 95%) and express high levels of the Fc-γ receptor CD16. Ex vivo $CD56^{dim}$ cells contain detectable perforin and thus are expected to mediate cytotoxicity. In contrast, the $CD56^{bright}$ NK cells express low levels of perforin but express high levels of cytokines and are thought to be an important inflammatory or regulatory subset (Orange and Ballas 2006). It is surprising that only a few attempts have been made to further characterize NK subsets distribution during stress. Thus J.A.

Bosch and coworkers (Bosch et al. 2005) reported that acute psychologic stress increased the number of cytotoxic CD56 ^{dim} NK cells without altering the immunoregulatory CD56 ^{bright} NK cell counts. Conversely, chronic stressors (as well as 1 month of intensive competitive sports training) caused an increase in the number of CD56^{bright} NK cells but no change in the number of CD56^{dim} NK cells (Suzui et al. 2004). At the same time, the examination of nine cosmonauts who flew 187–196-day missions aboard the ISS showed that the number of CD56^{dim} and CD56 ^{bright} NK cells (among CD16^{+}CD56^{+} and CD16^{-}CD56^{+} NK cells) was decreased during the first seven days after landing. These results suggest that space mission–associated factors can result not only in decreased level of circulating NK cells with a high cytotoxicity but also decreased level of the major cytokine-producing subset of NK cells.

It is well known that interleukin 2 activation of NK cells rapidly induces the expression of CD69 antigen (Lanier et al. 1988). This activation antigen represents a triggering surface molecule in NK cell clones as its stimulation triggers the cytolytic machinery of these cells (Borrego et al. 1993). Beyond its role in NK cell cytotoxicity, CD69 is involved in regulating other NK cell functions such as TNF-α production and the expression of other, functionally relevant, activation antigens, such as the inter-cellular-adhesion-molecule (ICAM)-1 by a mechanism that involves Ca^{2+} mobilization (Borrego et al. 1999). Expression of cell surface marker CD69 on NK cells, induced by IL-2, was assessed by performing whole blood cultures for 18 h in 10 cosmonauts before and after long-duration missions aboard the ISS. A marked decrease in the number of CD56^{+} NK cells expressing this marker was observed on the first and seventh days after landing. Such effect may reflect in part a decrease in the functional potential of NK cells.

Although studies such as those described above provide useful information on NK cell function after long-duration space missions, the main question whether the altered NK cells were already present during flight, or was caused solely by landing effects remains. Comparing results from brief and long flights may facilitate understanding of the differences and interrelationships between the multiple stressors associated with spaceflight and landing. In spite of substantial differences in methodology used, the results from American and Russian studies conducted before and after short-duration missions (4–16 days) showed decreased NK cell cytotoxicity, capacity of lymphocytes to bind target cells, and decreased number of NK cells after landing (Konstantinova and Fuchs 1991; Meshkov and Rykova 1995; Tipton et al. 1996; Mehta et al. 2001; Mills et al. 2001; Rykova et al. 2001; Borchers et al. 2002; Sonnenfeld and Shearer 2002; Crucian et al. 2008; Rykova et al. 2008). These data might suggest that landing stress (i.e., an acute response to reentry and readaptation to unit gravity) may be a major factor contributing to impaired NK functions of cosmonauts. On the other hand, in ground-based space analog studies, there was a significant decrease in total NK cell cytotoxicity during and after 120- and 360-day bed rest with head-down tilt (Konstantinova and Fuchs 1991). This observation indicates that functionally deficient NK cells may not result exclusively from landing stressors but could represent alterations in space flight. In addition, since experimental conditions of bed rest were the same for all subjects, the magnitude of changes

differed dramatically between individuals. This data underscores the importance of individual differences in responsiveness.

In conclusion, the mechanisms responsible for changes in NK cell activity during or after long-duration missions are not clear yet and seem to be the results of a combination of environmental and individual factors, hereby modulating multiple immunotropic hormonal pathways (e.g. catecholamines, glucorticoids, neuropeptides) (Meehan et al. 1993; Stowe et al. 2003). Future studies of the role of psychoneuroendocrine factors on spaceflight-induced alterations in NK cells as well as the interaction with the microbial load on board will expand significantly our understanding of the mechanisms of immune reactions as well as provide novel information about relationship of the immune system with other functional systems participating in adaptation of organism to altering exogenous and endogenous factors (see also Chaps. 8–10, 12–17).

We summarize that the past three decades of continuous research has revealed that spaceflight factors can suppress NK cell function of humans. The practical importance of these findings is indisputable, since these deviations indicate not only secondary immune-deficiencies that can increase the risk of diseases during long-duration space missions but also provide some evidence how the evolution of countermeasures seems to have affected NK cell functional properties and to increase its activities. However, the individual and the individuals' conditions are to be taken into account as well as the complex interaction with other immune cells and microbial antigens, and future and integrated research on-board space crafts need to be undertaken.

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Adaptive Immunity and Spaceflight

12

Brian Crucian and Clarence Sams

12.1 Adaptive Immune Cell Function

The distribution of the various immune cell subsets and their functional capacity are mutually exclusive considerations. Cellular distribution may be dramatically altered while cell functional capacity is normal. Alternatively, the distribution of peripheral immune cells may be unaltered, when functional capacity is reduced. Either a loss in cell numbers or a reduction in cell function may each separately result in a clinical immunodeficiency. Considering this, any assessment of immune status during spaceflight must include assays of both cell number and measures of cell function. There are many distinct assays to measure cell function. Some assays may be generalized and apply to several cell types, whereas others may be highly specific and apply only to a particular cell subset. Most functional innate immune assays consist of cell culture in the presence of a cellular stimulus, after which any event in the normal activation process may be monitored. For T cells, a specific sequence of cellular events, beginning within seconds and progressing over the course of days, leads to full activation and execution of an in-vivo response. Very early events (within minutes) include transmission of intracellular signals to the nucleus, protein kinase C (PKC) phosphorylation, and cytoskeleton reorganization. Over the course of hours there is expression of new mRNA and translation of new protein. Activation markers are expressed on the T cell surface at specific times postactivation, each with a specific purpose. CD69, preformed in the cytoplasm, is expressed within an hour, whereas CD25 (Interleukin (IL)-2 receptor) is expressed at about 24 h postactivation. A third marker of activation, HLA-DR (a MHC class II molecule), is expressed between 48 and 72 h postactivation. IL-2 is secreted, activating other T cells as well as the origination cell (autocrine stimulation). Depending on the local cytokine environment, antigen type/dose, and other factors, naive CD4 positive

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T-helper cells will proliferate, and develop into a variety of Th1, Th2, or Th17 cells. These cells will then produce additional effector cytokines to drive the adaptive immune response toward a particular bias, specific for the initiating pathogen or antigen. Cell division and expansion (proliferation) usually begins at around 72 h. Although any of the cellular events involved in T cell activation may be monitored as a functional assay, the expression of activation markers and production of cytokines are the most commonly used. These assays are relatively simple, and indicate if T cell activation has initiated, progressed, and resulted in effector function (cytokine production, etc.).

Representative studies, demonstrating specific adaptive immune functional alterations that have been observed during or immediately following spaceflight are summarized in Table 12.1. Several groups have observed that T cells in culture do not activate during spaceflight (or simulated microgravity) conditions. Specific findings include alterations in T cell activation characteristics, intracellular signaling, and other observable changes at the single-cell level (Cogoli 1997; Hashemi et al. 1999; Hughes-Fulford 2003). Microgravity culture was recently demonstrated to affect several T cell intracellular signaling pathways (see also Chap. 14) upstream of the protein kinase C (PKC), a family of enzymes involved in transuding cellular signals by controlling the function of other proteins. However phosphorylation of PKC was not down-regulated in T cells incubated in the presence of Concanavalin A (Con A) and anti-CD28 antibodies in simulated microgravity (Boonyaratanakornkit et al. 2005), and T cell stimulation with phorbol myristate acetate (PMA)+ ionomycin (direct PKC activation) was able to fully restore T cell activation during simulated microgravity (Cooper and Pellis 1998). This suggested that signaling pathways 'upstream' of PKC might be responsible for the 'microgravity defect' in normal T cell activation observed in microgravity. Hughes-Fulford et al. analyzed differential gene expression following T cell activation comparing normal gravity to simulated microgravity conditions, to identify gravity-sensitive intracellular signaling pathways. The study found that 99 genes were significantly up-regulated during early T cell activation in normal gravity; however during simulated microgravity the majority of these showed no mitogen-induced gene expression (Boonyaratanakornkit et al. 2005). Most of the gravity-sensitive genes were regulated by transcriptional factors NF-kB, CREB, Ets-like protein, AP-1, or STAT1. The authors concluded that gravity is a key regulator of cell-mediated immunity, and its absence slows or impedes signaling pathways required for early T cell activation.

While these microgravity cell culture measurements represent solid findings, it is unknown if these findings reflect a clinical risk for crewmembers. Since T cells from otherwise normal and healthy test subjects also do not activate during microgravity culture, these findings may potentially represent some gravisensing 'artifact' associated with the unique culture conditions. The in vivo situation is quite different from pure microgravity culture, with the addition of adhesion, tissue environment, pressure, motion, and shear flow variables. It is also possible that microgravity does indeed inhibit T cell function to some degree in vivo, thus explaining the in-flight DTH response data. However, additional crewmember variables such as stress and isolation make such mechanistic distinctions problematic.

Table 12.1 Representative studies: adaptive immune function and spaceflight

Category	Author	Flight phase	Findings
T cell intracellular signaling	Cogoli 1997	Review	T cells during reduced gravity, expression of IL-2 receptor is inhibited, cytoskeleton interactions with rap proteins are disturbed, the function of PKC is altered
	Hashemi et al. 1999	In	TCR stimulation during space flight: engagement and internalization, but T cells failed to express CD25. T cell intracellular signaling(likely PKC pathway) within first few hours of activation is disrupted
	Hatton et al. 2002	In	Microgravity differentially modifies protein kinase C isoform translocation in monocytes and T cells
T cell cytoskeletal rearrangement/ MTOC orientation	Lewis et al. 1998	In	Jurkats cells stimulated during spaceflight: diffuse shortened microtubules, poorly defined microtubule organizing centers (MTOC), and increased percentage of FAS/APO-1 apoptotic cells. Cytoskeletal changes potential cause of poor T cell activation during flight
	Hughes-Fulford 2003	In	Collapse of actin cytoskeleton, loss of focal adhesions, increase in PGE2 during microgravity. Actin and microtubule modification in microgravity, alteration in anabolic signal transduction, probably through growth factor receptors and associated kinase pathways connected to cytoskeleton
Leukocyte proliferation or blastogenesis	Konstantinova et al. 1973	Post	Human lymphocytes have decreased responses to mitogens following spaceflight
	Kimzey et al. 1975	Post	Marked decreased in mitogen-stimulated RNA and DNA synthesis by lymphocytes following both 28 days and 59 days spaceflight
	Cogoli et al. 1984	In	Mitogenic stimulation of cultured lymphocytes during spaceflight shows almost no proliferative/ DNA synthesis response
	Nash and Mastro 1992	Post	Following 4 day spaceflight, proliferation of lymph node lymphocytes to ConA or PMI-I depressed, not restored by exogenous IL-1 or IL-2. Response to PHA not decreased. Proliferation of splenocytes was not depressed
	Cogoli 1993	In	T lymphocyte response to mitogens depressed by average of 56% in humans during and following spaceflight (n = 129)
	Grove et al. 1995	Post	Following 10 day spaceflight, lymph node lymphocytes and splenocytes showed decreased IL-2 production in response to T cell receptor (TCR) independent mitogens, but unchanged following TCR stimulation
	Pippia et al. 1996	In	Addition of exogenous IL-1 does not restore loss of lymphocyte proliferative response during microgravity
	Sonnenfeld et al. 1998	Post	Flown rats: blastogenesis of spleen cells in responses to mitogen inhibited for dams, but not for pups
	Gridley et al. 2009	Post	Mice flown onboard Space Shuttle: splenocyte DNA synthesis significantly reduced in response to PHA stimulation

(continued)

Table 12.1 (continued)

Category	Author	Flight phase	Findings
T cell function (early blastogenesis)	Hashemi et al. 1999	In	Cell cultures performed during spaceflight: TCR stimulation does not result in early blastogenesis (CD69/CD25), phorbol ester bypass only partially restores an activity. TCR internationalization and monocyte function appear not to be responsible for T cell deficit
	Chouker et al. 2008	Post	T cell early blastogenesis depressed following long-duration spaceflight. Percentage of T cell subsets capable of being stimulated to secrete cytokines depressed following both short and long-duration spaceflight
Delayed type hypersensitivity	Taylor and Janney 1992		DTH responses diminished during short duration spaceflight. *N*=10; antigens administered 46 hours before landing, reactions read 2 hours following landing
	Gmunder et al. 1994		DTH responses dimimished during or following long-duration spaceflight onboard MIR space station in 4 of 5 subjects
Virus-specific T cell function	Stowe 2003		Levels of EBV viral specific T cells elevated prior to flight, their function reduced at landing
	Stowe 2009		Samples collected during short-duration spaceflight: EBV-specific T cell levels increased, their function is reduced
NK cell function	Konstantinova et al. 1995		Postflight, NK cell binding and lysis of target cells reduced, production of NK cells decreased
	Meshkov and Rykova 1995		Decrease in percentage of lymphocytes that can bind targets leads to reduction in total NK activity
	Buravkova et al. 2004		During cell culture onboard the ISS, NK cell binding and cytotoxicity to K562 target cells was unchanged

Removing and culturing cells from crewmembers or animals during or following spaceflight, and performing cell function assays using terrestrial cell culture, provides subject-specific clinical information. Almost universally, activation, blastogenesis, and proliferation are altered in crewmembers or animals postflight (Fig. 12.1). Since these cultures are free of the microgravity cell culture 'artifact', this must represent legitimate in vivo immunosuppression. It is likely that multiple factors may explain these observations. Certainly stress hormone levels associated with the high-G reentry and readaptation to unit gravity play a significant role. Elevated stress hormone levels are commonly observed postflight (Gmunder et al. 1994; Meehan et al. 1992; Mills 2001; Stowe 2003), and corticosteroids are known to suppress immune function. It is also possible that any microgravity effect on T cell function may linger, and thus require some 'recovery' time period. Unfortunately, minimal data exists from samples collected during spaceflight, and cultured terrestrially, to determine if these postflight observations reflect the in-flight condition. There are some limited studies that indicate adaptive immunity is in fact dysregulated

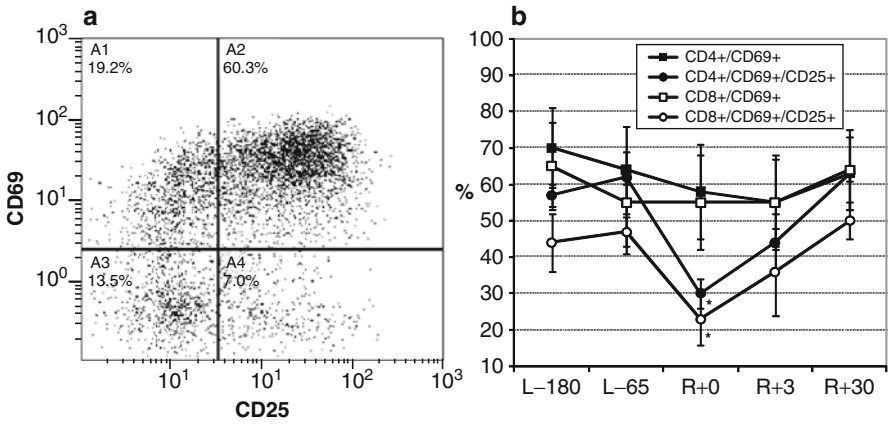

Fig. 12.1 (**a**) Normal T cell activation during cell culture consists of expression of CD69 on the T cell surface (within 1 h), followed by expression of CD25 (at about 24 h). (**b**) T cell activation is significantly reduced, for both CD4+ and CD8+ subsets, following short-duration space flight; "L-": days before launch, "R+" days after retrun to Earth

during spaceflight. Delayed type hypersensitivity responses were found to be blunted during long-duration mission (Taylor and Janney 1992; Gmunder et al. 1994). This assay, unfortunately no longer available, provided an excellent in vivo measure of immune function in human subjects. Latent herpes virus reactivation, an indirect indicator of loss of cytotoxic T lymphocyte functional control, occurs to high levels during spaceflight (see Chap. 16).

12.2 Alterations in Cytokine Production Profiles

In addition to the generalized functional measures described above, a primary end-effector function following activation of adaptive immune cells is the production of cytokines. Cytokines are the signaling molecules of the immune system, and are responsible for cellular cross talk, recruitment, and activation among immune cell subsets. They regulate the type and magnitude of any immune response. Cytokines generally possess a short half-life, and thus function at low concentrations in a localized environment restricting the reaction to the site of pathology. Upon binding, cytokines may upregulate expression of activation genes, induce new protein expression, and initiate proliferation and cellular effector function. CD4+ T cells are the primary cytokine producing cells within adaptive immunity, and essentially regulate adaptive immune responses between cellular and humoral, as well as pro- and anti-inflammatory biases. Recent advances in cytokine biology have identified several specific categories of T cell immune responses based on specific patterns of cytokine expression. These categories are Th1, Th2, and Th17 T cell responses, and generally result in monocytic, mast cell/basophilic, or neutrophilic patterns of inflammation, respectively.

These categories of cytokines generally inhibit each other's production, so only a single immune bias may be realized. Proper T cell cytokine regulation is important for immune health, and inappropriate biases (either hypo or hyper) may result in clinical disease. Rheumatoid arthritis is a Th17-mediated disease, whereas excessive Th2 responses can result in allergies and hypersensitivities (Jager and Kuchroo 2010).

Much may be learned about immune status and function by measuring cytokine levels following immune cell stimulation during cell culture. There have been many studies that have investigated cytokine production profiles associated with spaceflight. A summation of representative data regarding cytokines and spaceflight is presented in Table 12.2. As might be expected the spaceflight data vary considerably based on human vs. animal, vehicle/duration, mitogen used, culture system used, and other experimental variables. There are however, some generally common findings. The adaptive immune cytokine Interferon-g is found to be suppressed during/following spaceflight (Gould et al. 1987; Sonnenfeld 1988; Crucian et al. 2000, 2008), whereas production of inflammatory cytokines such as IL-1, IL-6, and IL-8 seem to vary associated with spaceflight (Chapes et al. 1994; Miller et al. 1995; Sonnenfeld et al. 1996; Kaur et al. 2008). There is a postflight decrease in the IFNg:IL-10 ratio and a Th1:Th2 shift during spaceflight (Crucian et al. 2008). A Th1:Th2 shift would seem to be supported by other evidence, such as diminished cell-mediated immunity during spaceflight (Taylor and Janney 1992; Gmunder et al. 1994), yet unaltered humoral immunity during spaceflight (Fuchs and Medvedev 1993; Stowe et al. 1999). In fact, Sonnenfeld summarized that cytokine changes in astronauts during spaceflight would not involve a general shutdown of the cytokine network, but rather involve selective alterations of specific cytokine functions (Sonnenfeld and Miller 1993). Either a generalized cytokine/functional immunosuppression, or a Th2 cytokine shift, would result in specific clinical risks for crewmembers should the dysregulation persist for the duration of exploration-class missions. Further in-flight assessments, or in-flight sampling followed by terrestrial analysis, using complete cytokine arrays will be required to completely understand cytokine dysregulation in-flight and derive associated clinical risks.

12.3 Humoral Immunity

Humoral immunity has not been investigated to the extent of which the cellular aspects of adaptive immunity have been investigated. Those studies which have been performed seem to indicate that humoral immunity may not suffer defects during spaceflight similar to cellular immunity. Following space flight, no changes were observed in total immunoglobulins and Ig isotypes for Russian cosmonauts (Fuchs and Medvedev 1993). Similar results were reported following Space Shuttle flights for US astronauts (Stowe et al. 1999), and in Russian Cosmonauts following long-duration flight onboard the International Space Station (Rykova et al. 2006).

Table 12.2 Representative studies: adaptive immune cytokines and spaceflight

Author	Flight phase	Human/ animal	Cells	Mitogen	Findings
Gould et al. 1987	Post, short	Rats	Splenocytes	Concanavalin A (Con-A)	IFNg production severely inhibited, IL-3 production unaffected
Sonnenfeld 1988	Post, short	Rats	Splenocytes	Con-A	IFNg production severely inhibited, IL-3 production normal
Chapes et al. 1994	In, short		Marrow macrophage line	LPS	Production of TNFa, IL-1, IFNb, IFNg elevated during spaceflight
Miller et al. 1995	Post, short	Rats	Splenocytes, thymocytes	Con-A or LPS	Both spleen and thymus cells secreted elevated IL-2, but only thymus cells secreted elevated IL-6
Sonnenfeld et al. 1996	Post, short	Rhesus monkey	Leukocytes		Production of IL-1 and expression of IL-2 receptor decreased after space flight
Crucian et al. 2000	Post, short	Human	Peripheral blood mononuclear cells (PBMC)	PMA/I	Intracellular production of IL-2 reduced in CD4 and CD8-positive T cells; production of IFNg reduced in CD4 T cells only
Kaur et al. 2008	Post, short	Human	Monocytes	LPS	Postflight, crew monocytes produced lower levels of IL-6 and II-1b, and higher levels of IL-1ra and IL-8 compared to control subjects
Crucian et al. 2008	Post, short and long	Human	Whole blood	Anti-CD3, PMA-I	Short duration: percentage of T cells secreting IL-2 and IFNg decreased, IFNg:IL-10 ratio decreased. Long duration: percentage of T cells secreting IL-2 reduced, secreting IFNg unchanged, IFNg:IL-10 ratio reduced
Gridley et al. 2009	Post, short	Mouse	Splenocytes	Anti-CD3	Decreased production of IL-2, increased production of IL-10, IFNg, MIP-1a following short-duration spaceflight
Fitzgerald et al. 2009	In	Human	Lymphoid tissue	Antigenic polyclonal	Cell culture on board ISS, lymphoid cells did not respond to antigenic or polyclonal stimulation, losing ability to produce antibodies or cytokines (cells stimulated before launch maintained their ability onboard ISS)
Gridley et al. 2009	Post, short	Mouse	Splenocytes	LPS	Secretion of IL-6 and IL-10, but not TNFa, increased in flown mice
Morukov et al. 2010	Post, short	Humans	PBMC	LPS+PHA	Alterations in Th1:Th2 cytokine production following space flight indicating a Th2 shift

12.4 Ground-Analog Studies

Despite the great success of the Space Shuttle, MIR and ISS programs, historically there has been extremely limited access to in-flight resources to support on-orbit studies. Limitations on up/down mass capability, crew time, power, volume, and microgravity-compatible instrumentation, have all precluded large-scale or complicated studies as may be readily performed on Earth. Some aspects of human spaceflight may be replicated to high fidelity using 'ground-based space analogs'. It is thus very advantageous when possible to advance spaceflight knowledge using these available analogs. Choice of analog depends highly on the physiological system of interest. For example, prolonged head-down tilt bed rest is an appropriate analog bone loss and muscle deconditioning. However, HDT bed rest does not replicate the likely causal factors of spaceflight-associated immune dysregulation: mission stress, isolation, confinement, disrupted circadian rhythms, microgravity, radiation, etc. The best likely terrestrial analog for these particular space factors would include extreme environment deployment, prolonged isolation, legitimate subject risk, and operational mission activities. For immune studies, NASA has investigated deployment to the Canadian arctic and undersea station deployment as human analogs for immune dysregulation. The Russian Space Agency has recently initiated the MARS500 project, a high-fidelity simulation for the confinement and duration of a projected Mars mission, which will include an immune surveillance component. The European Space Agency, using the French-Italian 'Concordia Station' at Dome C on the high Antarctic plateau, has initiated an ongoing study of immune and stress parameters during Antarctic winter over. The Antarctic analog, with prolonged 1-year mission deployment, extreme environment and risk, long travel, subject isolation, disrupted circadian rhythms, mission objectives, and station environment, likely represents one of the best human space analogs for immune dysregulation on Earth. This ESA study is ongoing, but will attempt to validate life at Concordia as a ground analog for spaceflight immune dysregulation (see also Chaps. 13 and 31, 32). Any validated analog could then be used for further mechanistic studies or evaluation of countermeasures.

Other nonhuman analogs exist which may have significant utility. Single cell studies may be performed in 'clinostat' or 'bioreactor' devices, which simulate microgravity during cell culture by slowly spinning the culture vessel, resulting in a constantly randomized gravity vector. Studies have indicated similar dysregulation of T cell activation during bioreactor culture as has been observed during on-orbit culture (Hashemi et al. 1999). Animal studies, offering a greater variety of sampling opportunities than human studies, may be performed using various stress models such as hind-limb suspension or confinement situations.

12.5 Conclusion

This chapter has summarized the current knowledge base regarding the function of the adaptive immune system and spaceflight. Although some of the postfindings are almost certainly related to landing and readaptation, it is likely at least some postflight

observations may reflect in-flight immune alterations. In fact, the very limited in-flight studies that have been performed do indeed confirm an adaptive immune system decrement during both short and long duration spaceflight. Lacking is a comprehensive in-flight immune assessment in humans during long-duration flight, to a comparable degree as has been successfully done in some ground-based space analog environments. This – together with ongoing and new earthbound and clinical studies – is required to clearly define human immunity during flight and draw conclusions related to the individual crewmember's clinical risk for disease. Should a severe in-flight dysregulation be found to persist during long-duration flight, as ongoing projects on the ISS are indicating ("Integrated Immune", "IMMUNO", and IBMP-Immune studies) and hence are not transiently related to either launch or landing, certain clinical risks would indeed result (Crucian and Sams 2009). If this were the case, both a monitoring strategy and countermeasures validation would be required prior to the initiation of exploration missions (Chouker et al. 2008).

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Stress, Hypoxia, and Immune Responses

13

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13.1 Introduction

Immune cells are unique in their capacity to distinguish between self and nonself. This ability is more or less pronounced with respect to cells of the adaptive and the innate part of the immune system, respectively. Although specificity of immune cells in targeting foreign microbial structures is well developed overall, damage to normal tissue may occur in excessive inflammatory reactions (Fig. 13.1). Under such conditions, overactive immune cells release a large spectrum of cytotoxic mediators causing damage to vascular endothelial cells, especially at the level of the microcirculation. As a result, perfusion-dependent oxygen delivery to tissues gets compromised and severe tissue hypoxia ensues. Hypoxia in turn gives rise to the stabilization of hypoxia-inducible factors and leads to the enhanced degradation of adenine nucleotides to adenosine. In search of physiologic mechanisms directed at the limitation of inflammatory collateral tissue damage during ongoing immune cell-mediated destruction of pathogens, a critical role for hypoxia in protecting tissues was revealed. Collateral tissue damage-associated deep hypoxia, hypoxia-inducible factors (HIF), and hypoxia-induced accumulation of adenosine may represent one of the most fundamental and immediate tissue-protecting mechanisms, with HIF-1 α, adenosine $A2_A$ and $A2_B$ receptors triggering "OFF" signals in activated immune cells. In these

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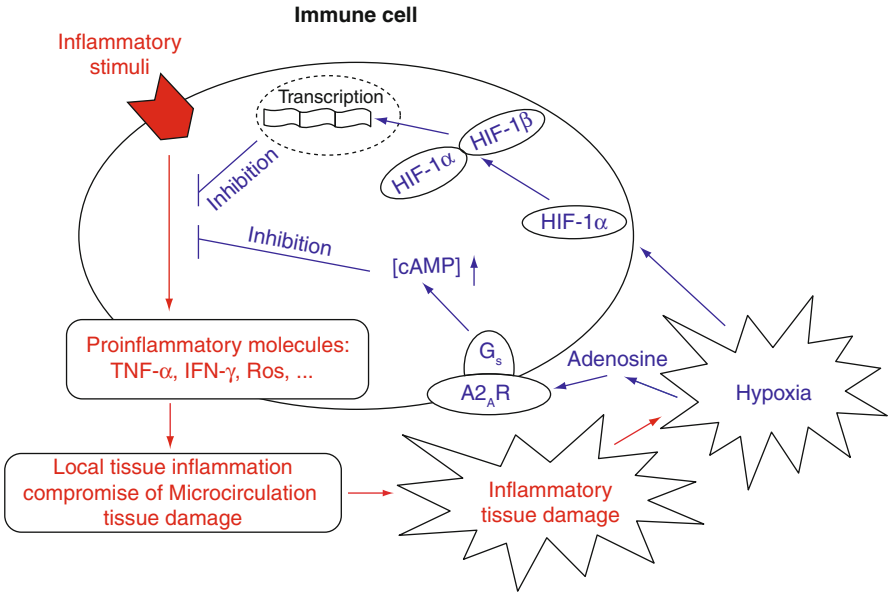

Fig. 13.1 Regulation of immune cells by inflammatory hypoxia: Under conditions of inflammation, immune cells may get overactivated and cause collateral tissue injury ending up in tissue hypoxia. Hypoxia triggers the breakdown of adenosine triphosphate (ATP) to form adenosine which acts in a delayed negative feedback manner. In this model, the extracellular adenosine serves both as a "signal" and the adenosine $A2_A$ receptors as "sensors" of excessive inflammatory collateral tissue damage. Adenosine $A2_A$ receptor activation will stimulate formation of intracellular cyclic adenosine monophosphate (cAMP), a well-known inhibitor of immune cell effector functions. In parallel, hypoxia will stabilize hypoxia-inducible factor (HIF-1α), also shown to counteract immune cell activation. These hypoxia-dependent signaling pathways help to counteract further immune cell activation and hence limit inflammatory collateral tissue injury. Although good in the short term, prolonged activation of these hypoxic mechanisms will lead to severe immunosuppression in the long term. (Modified from Sitkovsky et al. 2004)

regulatory mechanisms, oxygen deprivation and extracellular adenosine accumulation serve as "reporters," while HIF-1 α, adenosine $A2_A$ and $A2_B$ receptors serve as "sensors" of excessive tissue injury. HIF-1 α by not yet characterized signaling pathways and adenosine $A2_A/A2_B$ receptor-triggered generation of intracellular cAMP strongly inhibit activated immune cells in a delayed negative feedback manner to prevent additional tissue damage (Sitkovsky et al. 2004; Thiel et al. 2007).

Thus, short-term activation of hypoxia-dependent anti-inflammatory signaling mechanisms is considered to be beneficial in tissue protection from immune cells-mediated inflammatory collateral damage, while long-term activation of these anti-inflammatory mechanisms by chronic hypoxia may cause severe immunosuppression, predisposing an individual to microbial infections.

Although chronic exposure to stress has clearly been demonstrated to be associated with higher rates of infection (Dhabhar 2009), only very little is known about the role

of hypoxia-triggered immunosuppressive mechanisms in astronauts/cosmonauts during long-duration space flights (LDMs). This brief review focuses on questions of whether stress encountered by astronauts/cosmonauts (i) may trigger neurohumoral effector mechanisms leading to tissue hypoxia and/or (ii) may also cause up-regulation of the same hypoxia signaling-dependent anti-inflammatory pathways even in the absence of low tissue pO_2 tension, i.e. also under normoxic conditions.

13.2 Evidence for Hypoxia to Cause Immunosuppression

The hypoxia → adenosine → adenosine-receptor pathway is an evolutionary old mechanism to control inflammation. In this pathway adenosine acts by binding to widely distributed adenosine $A2_A$ and $A2_B$ receptors located on the cell surface (Linden 2001). Adenosine A2 receptors activate the enzyme adenylylcylase leading to the enhanced production of cyclic adenosine-monophosphate (cAMP). cAMP itself represents a very potent 'OFF' signal in activated immune cells. Although this sequence of events is part of a delayed negative feedback manner mechanism directed at the protection of tissues from uncontrolled ongoing excessive inflammation, down-regulation of immune cells may cause severe immunosuppression (reviewed in Linden 2006). Indeed there is a lot of in vivo and in vitro evidence for hypoxic-adenosinergic suppression of cells of innate and specific immunity. Although there are some reports on activation of distinct functions of phagocytes (e.g. up-regulation of ß2-integrins) upon short-term exposure to hypoxia (Scannell et al. 1995) or moderate hypobaric hypoxia (Hitomi et al. 2003), capacity of phagocytes to kill germs is severely impaired (Segal et al. 1981; Leeper-Woodford and Mills 1992). Similarly, production by macrophages - and T lymphocytes of chemotactic and activating factor or monocyte chemoattractant protein-1 (MCP-1) was inhibited during hypoxia (Bosco et al. 2004).

While T cell-mediated immune responses are suppressed, B-lymphocytes have been shown to still produce immunoglobulins under hypoxic conditions (Sitkovsky et al. 2004). Moreover, hypoxia resulted in a shift of T-helper cell responses from a Th1 type to a Th2 type. This effect is discussed to be mediated by adenosine leading to the inhibition of the differentiation of naïve CD4 T-cells into Th2 cells via adenosine A2 receptors (Panther et al. 2003). As a result of a lack of oxygen, activity of cells especially of those of the specific immune system is severely suppressed (Sitkovsky et al. 2004).

The effects of hypoxia on lymphoid and nonlymphoid tissues can be further aggravated by breathing air with an oxygen content of lower than 20.9 vol.%, because this will enhance the accumulation of extracellular adenosine. Accordingly, we and others (Decking et al. 1997) showed in in vivo experiments that adenosine blood concentrations significantly increased upon breathing 10–12% oxygen (Choukèr et al., unpublished). Altogether, adenosine signaling via $A2_AR$ and $A2_BR$ and accumulation of immunosuppressive intracellular cAMP in activated immune

cells (Sitkovsky 2003) inhibits intracellular pathways required for synthesis and secretion ofpro-inflammatory and cytotoxic mediators by immune cells, effects which will terminate the immune cells' effector functions and thereby down-regulate the immune response.

13.3 Are Astronauts at Risk to Develop Tissue Hypoxia?

Concerning this question, it is of interest to note that several stressful conditions encountered by astronauts/cosmonauts during space flight might cause hypoxia leading to the up-regulation of hypoxia-related immunosuppressive signaling pathways. During spaceflight multiple stressors take effect on the body's psychological and physiological stress response systems. These systems include neurohumoral control mechanisms (Fig. 13.2) which are old in nature and have been shaped by a long history of evolutionary processes on earth. As it was not foreseen by evolution for man to leave earth, it is hard to predict how these endogenous stress response supersystems may interact during LDM and what the long-term effects on the astronauts'/cosmonauts' health status may look like.

Despite a lack of data collected during the course of spaceflight missions, there is some evidence from earth-bound models of simulated microgravity for the development of tissue hypoxia or hypoxia-like disturbances in response to stress especially caused by microgravity and/or radiation.

The loss in gravity is associated with an upward shift of the body's blood volume toward both the thoracic cavity and the head, respectively. Such a state of central hypervolemia will elicit a multitude of adaptive responses at the macro- and microcirculatory level (Vorob'ev 2004). Additionally, low or zero G conditions will significantly change airway breathing characteristics. Both altered central blood volume distribution and mechanical airway characteristics might end up in pulmonary ventilation/perfusion mismatch thereby compromising lung gas exchange. Such simple mechanistic effects are likely to be even more pronounced during sleep when physical activity and consciousness are decreased and control of airway patency is impaired (Prisk 1998).

At the microcirculatory level, oxygen delivery may be further limited by peripheral vasoconstriction due to elevated blood levels of stress hormones like vasopressin, adrenaline, and noradrenaline, with the action of the catecholamines being potentiated by vasoconstrictor permissive effects of increased blood concentrations of cortisol (Yang and Zhang 2004). Tissue malperfusion may additionally be aggravated by partial obstruction of capillaries due to catecholaminergic activation and aggregation of blood platelets, known to be induced upon stimulation of $\alpha 2_a$ adrenergic receptors located on the surface of thrombocytes (Tschuor et al. 2008.).

In this scenario, a decrease in rheological properties of erythrocytes due to decreased cell membrane fluidity and increased cell stiffness will further add to tissue malperfusion. Such effects are even more pronounced by increased hematocrit values which are frequently observed in astronauts/cosmonauts during LDMs. The rise in hematocrit values, however, is not due to an increase in red cell mass, but results from its decrease which is paralleled by a much stronger restriction of the

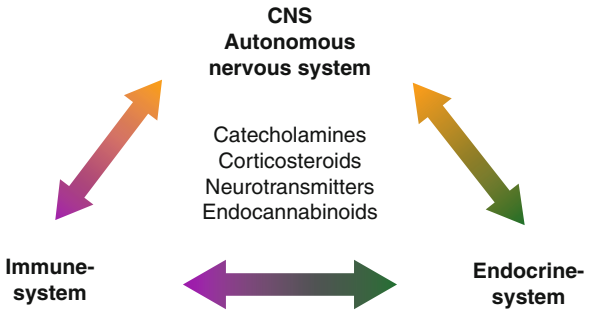

Fig. 13.2 Stress Supersystems: The major stress supersystems of the body comprise the sympathetic and parasympathetic parts of the autonomous nervous system, the endocrinological hypothalamic-pituitary-adrenal gland axis and the immune system which mutually interact in a highly compartmentalized and timely coordinated fashion to cope with psychological and physical challenges

plasma volume. In consequence, during LDMs circulating blood volume is in the end far below from normal in astronauts/cosmonauts which may predispose them to substantially increased risk for tissue hypoxia in case of unexpected cardiovascular or pulmonary challenges (Talbot and Fisher 1986).

Pathophysiological hypoxia elicited by microgravity is further aggravated in astronauts/cosmonauts by breathing even normoxic or hypoxic air mixtures at hypobaric or normobaric pressures, respectively. Such environmental stressors are likely to be encountered during space exploration missions when air pressure and oxygen content will be reduced in Lunar or Martian living habitats to reduce risks of fire or to facilitate extrahabitat activities in hypobaric space suits.

13.4 Are There Environmental Stress Conditions Which Are Able to Up-regulate Immunosuppressive Hypoxic Signaling Pathways Irrespective of Tissue Hypoxia?

Little is known about factors able to up-regulate or to mimic hypoxic signaling pathways independently from a lack in oxygen, i.e. under conditions of normoxia. In this regard stress caused by cosmic radiation (see Chaps. 17 and 25) likely represents an important one encountered by astronauts/cosmonauts during LDMs.

For instance, tumors respond to radiation especially upon reoxygenation in the postradiation phase by the nuclear accumulation of HIF-1 α. Enhanced translation of HIF-1 α-regulated transcripts, however, are very likely to compromise the efficacy of antitumor therapies by stimulation of tumor angiogeneic processes or by the suppression of cytotoxic functions of tumor infiltrating T-cells (Moeller et al. 2004.). These data describe novel pathways contributing significantly to our understanding of HIF-1α regulation by radiation which may be one of the major reasons for the development of immunosuppression in astronauts/cosmonauts during LDMs.

Accordingly, HIF-1 α was shown not only to directly suppress immune-stimulatory cytokine production but also to modulate key enzymes for the generation of

immunosuppressive adenosine. For instance, HIF-1 α up-regulates the AMP degrading and adenosine forming enzyme ecto 5′-NT (CD73) (Synnestvedt et al. 2002)and increases adenosine $A2_B$ receptor expression (Kong et al. 2006) due to the location of hypoxia-responsive elements (HRE) in the promoter region of these genes. Moreover, HIF-1 α by inhibiting adenosine kinase expression (Morote-Garcia et al. 2008) strongly attenuates the removal of formed adenosine via inhibition of its rephosphorylation to AMP. This will greatly increase levels of adenosine upon the release of ATP into the extracellular space and strengthen immunosuppressive signaling via A2 receptor expression (Sitkovsky et al. 2004). Such mechanism may be further augmented by the ability of radiation to cause direct release of ATP, the mother molecule for the generation of adenosine. A hypothesis under investigation is that opening of multidrug resistance protein P-glycoprotein 1, a MDR ATP/chloride channel, in response to increased local concentrations of peroxides may be one initial response to ionizing radiation. Notably, a rapid release of ATP was detected from cells irradiated with doses as low as 10 centigray (cGy) (Abraham et al. 1994). As the magnitude of ATP release correlated with the level of MDR1 expression, ATP release was rather mediated by the specific P-glycoprotein transmembrane channel than by nonspecific membrane leakage.

The direct effects of radiation in terms of enhanced ATP release and immunosuppressive adenosine formation are likely to be strengthened by other stressors known to cause ATP release from cells via activation of ATP-permeable channels and/or exocytosis of ATP-containing vesicles. Accordingly, ATP is set free from the intracellular pool upon challenge of cells by numerous stressors, like oxidative stress (Ahmad et al. 2006), mechanical stress (Qin et al. 2008), heat and osmotic stress (Kimura et al. 2000).

Most importantly, ATP is released into the synaptic cleft via exocytosis as a chemical cotransmitter of noradrenaline or acetylcholine neurotransmission following activation of the autonomous sympathetic and parasympathetic nervous system (Abbracchio et al. 2009). This is of potential immunomodulatory, immunosuppressive significance and may represent a direct link between psychic stress, activation of the autonomous nervous system, and direct immunosuppressive action of adenosine, since primary as well as secondary lymphoid tissues are directly innervated by sympathetic and cholinergic nerve fibers (Bellinger et al. 2008).

13.5 Is There Direct Evidence for Activation of Hypoxic Signaling Pathways in Astronauts/Cosmonauts?

Despite the clearly identified risk for the development of tissue hypoxia during LDM, to the best of our knowledge there are no space in-flight data available regarding tissue oxygenation in astronauts/cosmonauts. As a consequence it is also not known whether LDMs induce the aforementioned anti-inflammatory hypoxic signaling pathways which were shown by us to significantly contribute to immunosuppression.

Most studies on tissue oxygenation therefore arise from earth-bound models. For instance, indirect evidence for local circulatory hypoxia has been reported in

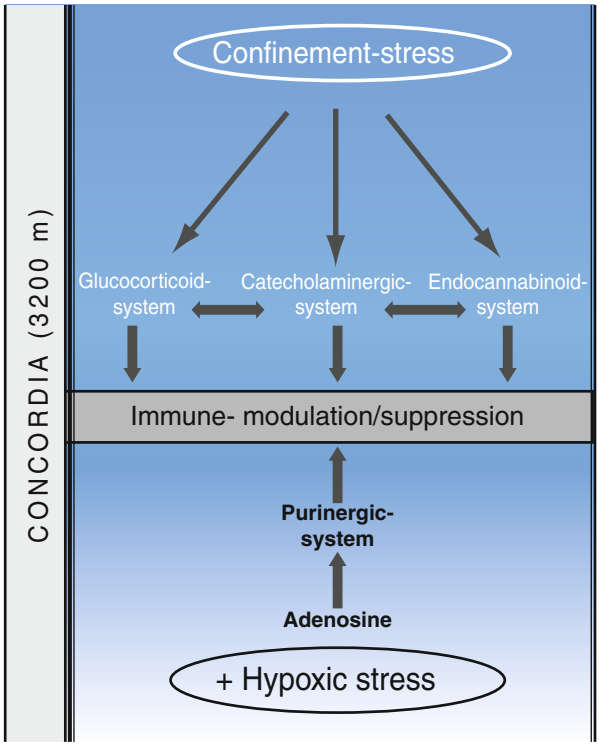

Fig. 13.3 CHOICE-Study: *C*onsequences of both long-term confinement ("Confinement Stress") and hypobaric *h*ypoxia ("Hypoxic Stress") *o*n *I*mmunity in the Antarctic *C*ONCORDIA *E*nvironment. The effects of hypobaric hypoxia (equivalent to 12–13 vol.% of O_2) will be controlled by the study of a group of age and sex-matched individuals living for a comparable period of time at sea level at the German Neumayer III base (~20 vol.% of O_2)

individuals subjected to head-down tilt to mimic microgravity-induced cardiovascular changes on central blood volume (Vorob'ev 2004). Accordingly, after 1 week in head-down tilt, local O_2 utilization, for example, by the arm was found to be increased as O_2 content was lowered and lactate increased in peripheral venous blood returning from the periphery, while respective parameters and mean pressure were unchanged in arterial blood. Besides such short-term experiments and other numerous observational studies strongly suggesting that astronauts/cosmonauts are prone to hypoxia (Grigor'ev et al. 2008) and immunosuppression (Guèguinou et al. 2009) during long-term spaceflight missions, long-term isolation experiments are currently run in the Antarctic region to determine how hypobaric hypoxia might affect the complex interaction between the body's stress supersystems at the psychoneuroendocrinological and immunological level (Fig. 13.2). In the *CHOICE*-Study (*C*onsequences of long-term-*C*onfinement and *H*ypobaric Hyp*O*xia on *I*mmunity in the antarctic *C*oncordia *E*nvironment) the stress effects caused by long-term confinement will be delineated from those elicited by hypobaric hypoxia. This can be achieved by the comparison of results obtained either at the Concordia base located at high altitude to the data gathered from crew at the Antarctic Neumayer III Station at the sea level (Fig. 13.3, see also Chap. 31). As oxygen tension is a major variable affecting any cell's function and hereby impacting health and immunity, investigations on the long-term effects of hypoxia in a confined and hostile environment as seen in Antarctic are becoming of increasing significance

for future space mission beyond the ISS when low oxygen pressure can become a prerequisite for extraterrestrial space habitats. Therefore not only the combination of hypoxia and confinement stress have been investigated but are currently extended by experiments designed to test also for the effects of a combination of hypoxia with conditions of bed-rest, the latter mimicking the space mission-relevant conditions of unloading of the human body.

In summary, the knowledge on stress-triggered, hypoxia-signaling-dependent anti-inflammatory and immunosuppressive mechanisms can be of critical importance for long-duration space mission and part of unfavorable impact on immune functions. The hypoxic and normoxic pathways of the neurohumoral stress response may synergistically end up in clinically relevant immune modulation. This knowledge about the interactions of stress- and hypoxia-sensitive pathways, as has been gathered in different condition, in space and in space analog studies, as well as from clinical and experimental models, will greatly benefit patients on Earth in the fields of surgery, emergency, and intensive care medicine. This all the more as in the hostile environment of an Intenisve Care Unit critically ill patients experience substantial amounts of psychological and physical stress frequently complicated by tissue hypoxia.

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Gravitational Force: Triggered Stress in Cells of the Immune System

14

Oliver Ullrich and Cora S. Thiel

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

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

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

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

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

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

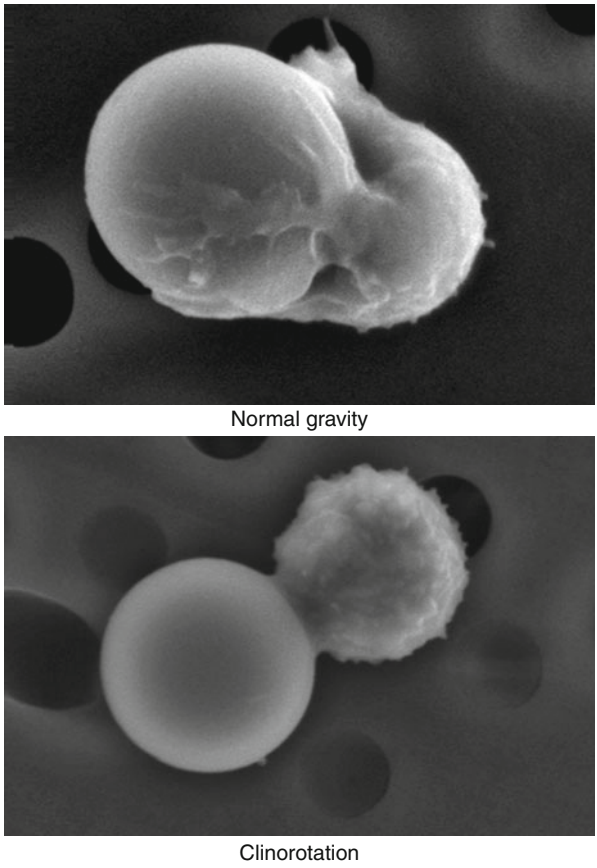

Fig. 14.1 T-cell activation in 1g (static) culture versus simulated microgravity (clinorotation); Photo credit to Clarence Sams (NASA Johnson Space Centre) and Mayra Nelman-Gonzalez (Wyle)

14.2 Gravisensitivity in Cells of the Immune System

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

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

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

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

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

14.3 Cell Migration and Cytoskeletal Architecture in Altered Gravity

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

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

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

14.4 Learning About Graviperception in Unicellular Systems

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

14.5 The Question of Sensing Gravitational Forces in Human Cells

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

14.5.1 Sensing Stress as Changes in the Gravitational Environment

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

14.5.2 Cellular Mechanosensory System

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

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

14.5.3 Force-Sensitive Ion Channels

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

14.6 Conclusion

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

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

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

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

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

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

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Microbial Stress: Spaceflight-induced Alterations in Microbial Virulence and Infectious Disease Risks for the Crew

15

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Although preventative measures to mitigate infectious disease risks to the crew are stringently enforced prior to the launch of spacecraft, pathogenic organisms are still carried by crewmembers, the spacecraft, and its cargo (Taylor 1974; Castro et al. 2004; Gueguinou et al. 2009) (see also Chap. 21). Of additional concern is spaceflight food, which is randomly monitored for microbial content prior to flight, yet remains a potential route of infection for food-borne pathogens, such as *Salmonella* sp. and *Staphylococcus aureus*. While the crewmembers are exceptionally healthy, dysfunction of their immune system has been repeatedly associated with spaceflight missions (Gueguinou et al. 2009), suggesting an increased susceptibility to infection. Other factors, such as the relatively crowded living conditions on flight vehicles, also increase the risk of infectious disease during spaceflight.

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Indeed, transfer of microbial flora between crewmembers has been demonstrated (Taylor 1974; Pierson et al. 1996). In addition, infections from the astronauts' own normal microbiological flora are still a risk, such as staphylococcal and streptococcal skin infections and urinary tract infections. Evaluations of the microbial ecology aboard Mir and the International Space Station (ISS) indicated a predominance of common members of the environmental flora (Castro et al. 2004), although the appearance of medically significant organisms has been documented (Ott 2004). Moreover, increased antibiotic resistance for some bacteria during culture in spaceflight has been reported (Tixador et al. 1985; Kacena and Todd 1999), which could potentially compromise effective prophylatic treatment if a crew member were to acquire an in-flight infection from such an organism. Latent viruses also remain a risk to the astronaut crews (see Chap. 16) because of their ubiquity, the ineffective current preventive practices (e.g., quarantine), and their immunocompromised state (Pierson et al. 2007). Thus, the presence of opportunistic and obligate pathogens and the corresponding risk of infectious diseases cannot be completely prevented during spaceflight.

In order to fully understand the impact of spaceflight on infectious disease risks to the crew, it is critical to advance our knowledge of the effects of spaceflight on the human immune system in a synergistic approach with studies to characterize spaceflight-associated changes in microorganisms and the resulting impact on the host–pathogen interaction. A wide variety of spaceflight experiments have been performed over the past 50 years demonstrating an extensive range of observed phenotypic and, recently, molecular genetic changes in microorganisms (Dickson 1991; Nickerson et al. 2004; Klaus and Howard 2006); however, information elucidating the mechanism(s) behind these changes and how spaceflight affects microbial virulence has only recently begun to emerge.

15.1 Modeling Aspects of Spaceflight Culture on Earth

Our knowledge of spaceflight-induced alterations in microbial virulence has been enhanced by the use of ground-based spaceflight analogue culture systems, such as the NASA-designed Rotating Wall Vessel (RWV) bioreactor (Fig. 15.1). The RWV is an optimized form of suspension culture in which cells are grown in cylindrical bioreactors, called high aspect ratio vessels (HARV) or slow turning lateral vessels (STLV) in physiologically relevant low fluid-shear conditions. The RWV consists of a hollow disk (HARV) or cylinder (STLV) that is completely filled with culture medium and rotates on an axis parallel to the ground (Klaus 2001; Nickerson et al. 2004). The result is solid-body rotation of the medium and the cells within and a constant rotation normal to the gravitational field that results in an environmental culture, which mimics aspects of the spaceflight environment (Nickerson et al. 2004). As a result, sedimentation of cells due to gravity is offset by the forces of the RWV rotation. The culture environment experienced by cells in the RWV is commonly

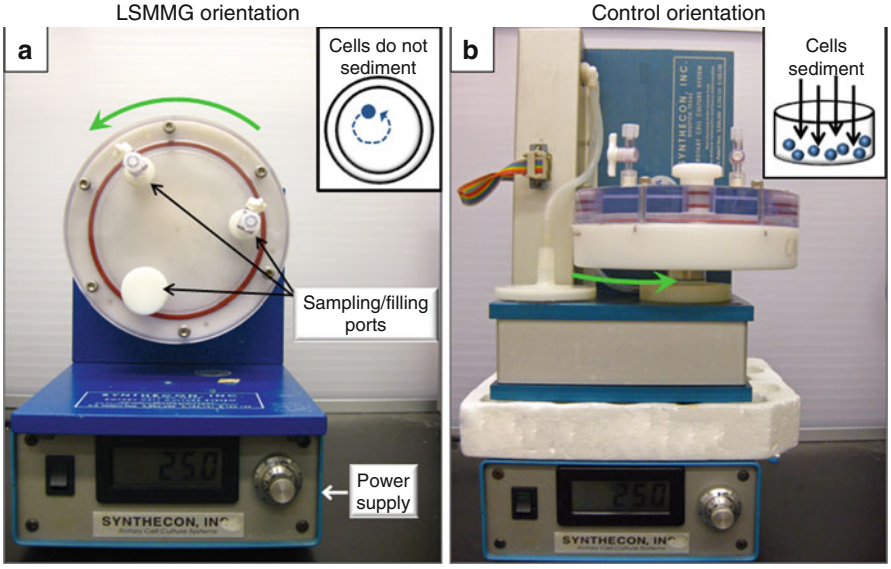


Fig. 15.1 *The rotating wall vessel (RWV) bioreactor and power supply.* The RWV bioreactor is depicted in the (**a**) LSMMG and (**b**) control orientations. For both orientations, the cylindrical culture vessel is completely filled with culture medium through ports on the face of the vessel (indicated by *black arrows* in **a**) and operates by rotating around a central axis. Cultures are aerated through a hydrophobic membrane that covers the back of the reactor. In the LSMMG orientation (**a**), the axis of rotation of the RWV is perpendicular to the direction of the gravity force vector. In the control orientation (**b**), the axis of rotation is parallel with the gravity force vector. The direction of rotation is indicated by a *green arrow* in both orientations. The effect of RWV rotation on particle suspension is depicted for each orientation (insets). When the RWV is not rotating, or rotating in the control orientation, the force of gravity will cause particles in the apparatus to sediment and eventually settle on the bottom of the RWV (**b**, *inset*). When the RWV is rotating in the LSMMG position, particles are continually suspended in the media (**a**, *inset*). The result is a solid-body rotation of the medium and cells within the RWV, with the sedimentation of the particles/cells due to gravity being offset by the upward forces of rotation. The result is low fluid-shear aqueous suspension that is similar to what would occur in true microgravity

referred to as low shear modeled microgravity (LSMMG), modeled microgravity (MMG), or simulated microgravity (SMG). Interestingly, the low fluid-shear culture conditions in the RWV are relevant to those encountered by numerous microbial pathogens and commensals during their normal life cycles in the gastrointestinal, respiratory, and urogenital tracts (Nauman et al. 2007). A gas-permeable membrane on one side of the RWV (HARV) or a central core gas exchange membrane (STLV) allows constant air exchange during growth (Nickerson et al. 2004). Several studies culturing microbes in both the RWV and true spaceflight have focused on profiling molecular genetic (transcriptomic and proteomic), phenotypic (*in vitro* stress), and virulence responses (*in vivo*) to provide new insights into how microorganisms

respond to culture in the microgravity environment of spaceflight. Notably, since pathogens encounter similar low fluid-shear regions in the human body, these studies have also revealed novel virulence strategies used by pathogens during the natural course of infection, and thus hold promise for the development of new strategies for treatment and prevention of infectious diseases on Earth.

15.2 Spaceflight Analogue (LSMMG) Culture of *Salmonella Enterica* Serovar Typhimurium

The first studies to demonstrate that culture of microbes in both LSMMG and true spaceflight conditions alters microbial virulence was performed using the obligate bacterial pathogen, *Salmonella enterica* serovar Typhimurium (S. Typhimurium) in a murine model of infection (Nickerson et al. 2000; Wilson et al. 2007). As a common food borne pathogen, S. Typhimurium was chosen as the model organism for these studies because (1) it has been extensively studied and well characterized, (2) it is a leading cause of intestinal and diarrheal disease in healthy individuals, and serious systemic illness in the immunocompromised, and (3) it is one of the five basic categories of organisms targeted by NASA for preflight monitoring of spaceflight food. Cultures of S. Typhimurium grown in the RWV environment (LSMMG) (Nickerson et al. 2004) displayed a significant increase in virulence as evidenced by a decreased time-to-death, decreased LD_{50}, and increased tissue colonization (liver and spleen) in murine infections as compared to control cultures. The *S.* Typhimurium LSMMG cultures also displayed increased survival in cultured macrophages and increased resistance to acid stress, two key pathogenesis-related responses that are relevant to bacterial virulence. In addition, this study was the first to demonstrate that the LSMMG environment elicits a global molecular genetic response in bacteria using 2-D protein gel electrophoresis to show that *S.* Typhimurium protein levels changed during LSMMG culture as compared to controls (Nickerson et al. 2000). This study established the paradigm that LSMMG can increase bacterial virulence and serve as a master signal to alter bacterial gene expression. Moreover, this work provided the first evidence that fluid-shear levels relevant to those encountered by *Salmonella* between the brush border microvilli of intestinal epithelial cells within the infected host act as a novel environmental signal that regulates the virulence, stress resistance, and gene expression of this pathogen (Nickerson et al. 2000, 2004).

To identify the *Salmonella* genes that changed expression in response to LSMMG culture, whole genome microarray analysis was performed using RNA harvested from *S.* Typhimurium cultures grown in LSMMG and control conditions (Wilson et al. 2002b). The results demonstrated that 163 genes globally distributed across the *S.* Typhimurium genome are either up-regulated (97 genes) or down-regulated (68 genes) during growth at LSMMG. These genes belonged to a variety of functional groups including protein secretion systems, lipopolysaccharide (LPS)

synthesis, ribosomal subunits, starvation/stress response, virulence factors, transcriptional regulation, iron-utilization enzymes and several of unknown function. Interestingly, none of the up-regulated genes corresponded to known virulence factors, even though LSMMG enhanced *S.* Typhimurium virulence. This suggests that LSMMG may alter *Salmonella* virulence by a previously uncharacterized mechanism(s) that could involve novel virulence functions. Alternatively, the increase in *Salmonella* virulence due to LSMMG may be the result of contributions of multiple genes of different functions that are regulated as part of the global reprogramming of *Salmonella* under LSMMG conditions. Secondary assays including RT-PCR and LPS gels were used to confirm the hits obtained from the microarray analysis. In addition, since the authors noticed that ferric uptake regulator (Fur) protein binding sites were associated with many of the genes found in the analysis, they tested the ability of an *S.* Typhimurium *fur* mutant strain to increase acid stress resistance under LSMMG conditions (as previously observed with the wild type strain in these conditions). The *fur* mutant did not display this phenotype, thus indicating that the *fur* gene may play a role in the response of *S.* Typhimurium to LSMMG.

Given the global alterations in molecular genetic and phenotypic responses of *S.* Typhimurium to LSMMG culture, it was hypothesized that the *rpoS* gene was a likely candidate for playing a role in LSMMG signal transmission, as it is a master regulator of the stress and virulence responses in many bacteria (Hengge-Aronis 2000; Dong and Schellhorn 2010). Specifically, an *S.* Typhimurium strain containing an *rpoS* mutation was extensively and systematically compared to an isogenic wild type strain for responses to LSMMG culture (Wilson et al. 2002a). This study provided key information regarding the bacterial LSMMG response: (1) the *rpoS* gene is not required for *S.* Typhimurium to display LSMMG-induced phenotypes (analyzed in exponential phase of growth), (2) LSMMG alters resistance to other stresses besides acidic and intracellular macrophage survival, including osmotic, thermal, and oxidative stress, and (3) cells grown in LSMMG in minimal media show a shorter lag phase and doubling time compared to control cultures.

A follow-up study demonstrated a progressive relationship between the applied fluid-shear in the RWV bioreactor and pathogenesis-related molecular genetic and phenotypic responses of *S.* Typhimurium (Nauman et al. 2007). When exposed to progressively increasing fluid-shear levels in the RWV, planktonic cultures of *S.* Typhimurium displayed corresponding progressive changes in acid and thermal stress responses and targeted gene expression profiles, including *rtsA*, a regulatory protein implicated in *Salmonella* intestinal invasion. This was the first study to provide evidence that incremental changes in fluid-shear can cause corresponding changes in biological responses in *S.* Typhimurium during the infection process and may lead to the discovery of new targets for antimicrobial therapeutic development against *Salmonella* infection as well as other cultures of planktonic bacteria.

15.3 Spaceflight Culture of *Salmonella Enterica* Serovar Typhimurium

To determine whether the true microgravity environment of spaceflight alters bacterial virulence and gene expression in a similar manner to that of spaceflight-analogue (LSMMG) culture, a flight experiment, designated as MICROBE, was flown aboard Space Shuttle mission STS-115 (Wilson et al. 2007). MICROBE was the first experiment to examine the effect of spaceflight on the virulence of a pathogen, and the first to obtain the entire molecular genetic response (transcriptomic and proteomic) of a bacterium to spaceflight. In this experiment, split samples of *S.* Typhimurium were grown in otherwise identical environmental conditions aboard the Shuttle during spaceflight and on the ground in the Orbital Environmental Simulator (OES) room at the Kennedy Space Center. Growth of *S.* Typhimurium was initiated in both settings after the Shuttle was established in microgravity conditions of orbit. A portion of the *S.* Typhimurium spaceflight cultures were preserved with fixative on orbit to preserve samples for RNA/protein analysis to measure gene expression changes (via microarray and proteomic assays); while the other portion of cultures were supplemented with fresh media and used (upon return to ground) for murine infections to measure virulence. Remarkably, the virulence assay results mimicked what was observed in LSMMG conditions in that the spaceflight cultures displayed increased virulence as measured by (1) decreased time to death, (2) decreased LD_{50}, and (3) increased percent mortality across multiple infectious dosages (given perorally) in murine infections as compared to ground controls (Fig. 15.2). In addition, 167 *S.* Typhimurium transcripts and 73 proteins were identified to change expression in response to spaceflight, and these genes were globally distributed across the *S.* Typhimurium genome and belonged to a variety of functional groups. Of the genes identified in microarray analysis, a preponderance belonged to the Hfq regulon (including those encoding small regulatory RNAs, outer membrane proteins, ribosomal proteins, stress response proteins, plasmid transfer functions, iron metabolism, and ion transport) as well as the *hfq* gene itself (which was down-regulated) (Table 15.1). Hfq is a highly conserved bacterial RNA chaperone protein that binds to small regulatory RNAs thereby facilitating their association with mRNAs, the result of which plays a diverse role in global regulation of prokaryotic gene expression, virulence, and physiology in response to stress (Gottesman 2004; Majdalani et al. 2005; Gottesman et al. 2006; Guisbert et al. 2007; Pfeiffer et al. 2007; Sittka et al. 2007, 2008). The spaceflight-induced Hfq regulon gene changes were up- or down-regulated in correlation with a decrease in *hfq* gene expression. This finding corroborates previous microarray analysis during *S.* Typhimurium culture in LSMMG, where the *hfq* gene is also down-regulated (Wilson et al. 2002b). Moreover, the number of down-regulated genes (98) was larger than the number of up-regulated genes (69) in response to spaceflight, another similarity to the LSMMG microarray results. Interestingly, Hfq also regulates expression of the Fur protein, which was found to play a role in

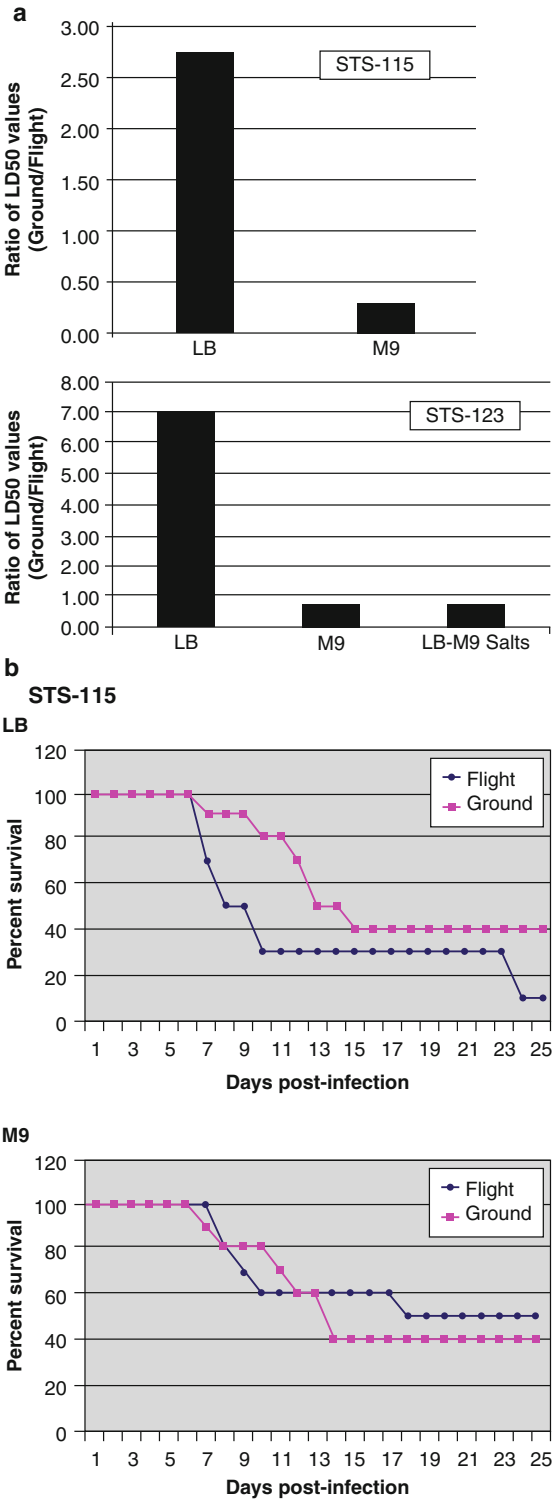
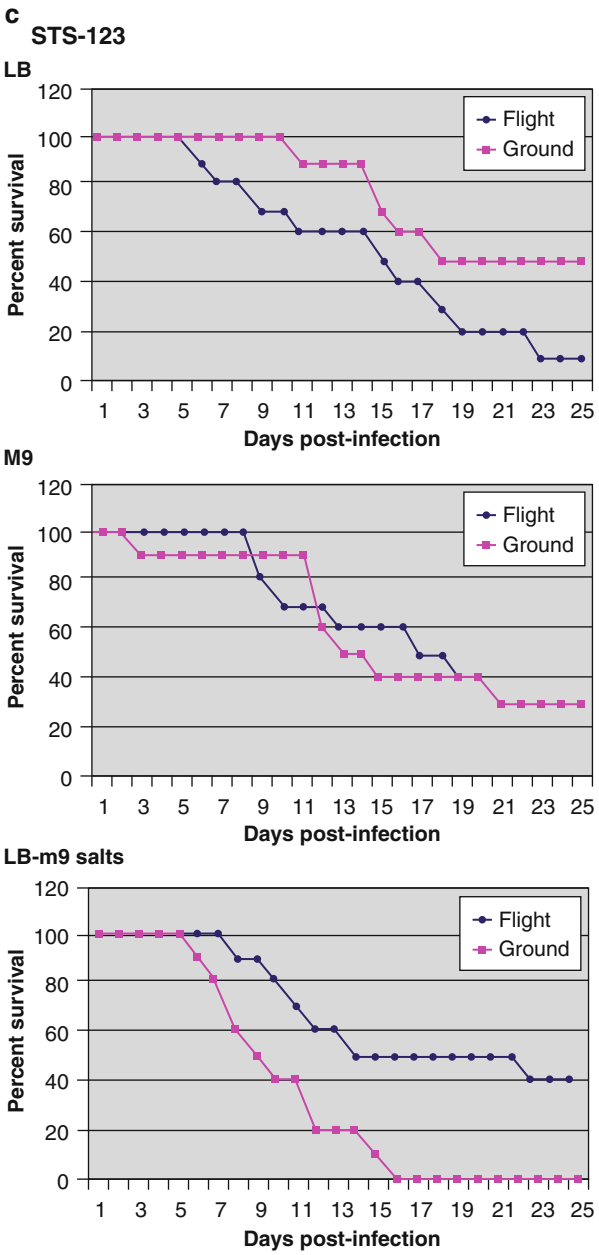


Fig. 15.2 *S.* Typhimurium *virulence in LB, M9 and LB-M9 spaceflight cultures.* (**a**) Ratio of LD_{50} values of *S.* Typhimurium spaceflight and ground cultures grown in LB (Lennox Broth), M9, or LB-M9 salts media from STS-115 and STS-123. Female Balb/c mice were perorally infected with a range of bacterial doses from either spaceflight or ground cultures and monitored over a 30-day period for survival. (**b**) Time-to-death curves of mice infected with spaceflight and ground cultures from STS-115 (infectious dosage: 10^7 bacteria for both media). (**c**) Time-to-death curves of mice infected with spaceflight and ground cultures from STS-123 (infectious dosage: 10^6 bacteria for LB and 10^7 bacteria for M9 and LB-M9 salts). Infectious dosages were selected such that the rates in time-to-death facilitated normalized comparisons across the different media. (**d**) SEM of spaceflight and synchronous ground control cultures of *S.* Typhimurium bacteria showing the formation of an extracellular matrix and associated cellular aggregation of spaceflight cells (magnification: × 3,500)

Fig. 15.2 (continued)



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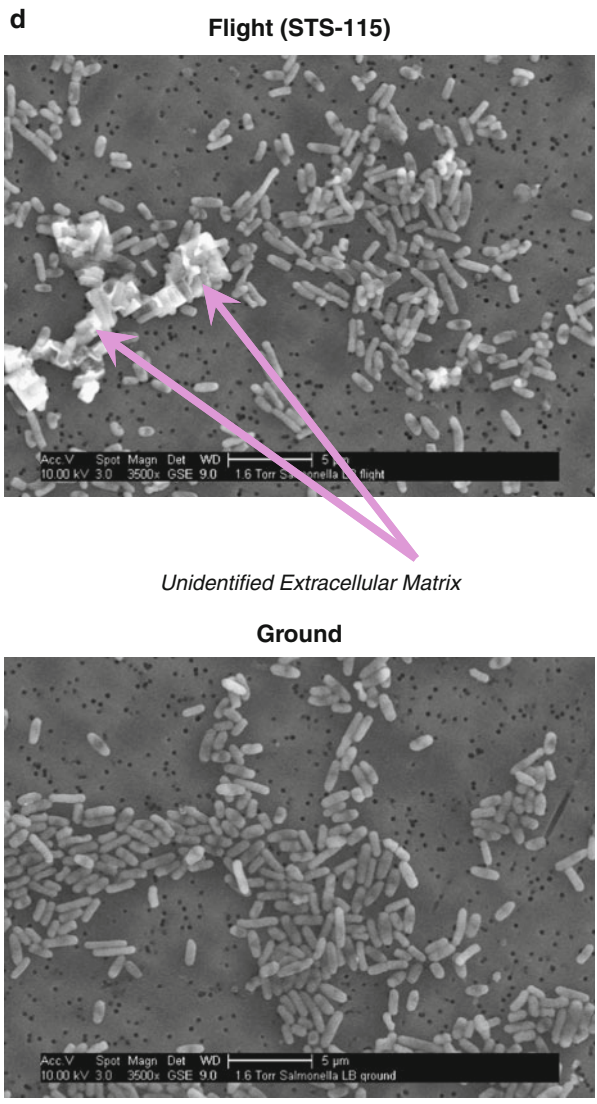

the LSMMG-induced acid stress response in *S.* Typhimurium. Subsequent LSMMG ground-based studies using an isogenic *hfq* mutant strain of *S.* Typhimurium not only supported involvement of Hfq in the *S.* Typhimurium response to microgravity, but they also established the utility of using the RWV in the laboratory to confirm observations obtained from spaceflight experiments. Interestingly, electron microscopic evaluation of *S.* Typhimurium spaceflight samples revealed striking

Table 15.1 Genes of the Hfq-regulon of *S.* Typhimurium and *P. aeruginosa* differentially expressed in response to spaceflight culture

Salmonella spaceflight			*Pseudomonas* spaceflight		
Gene number	Gene name	Function	Gene number	Gene name	Function
Stress resistance/virulence					
STM0831	*dps*	Stress response protein	PA2300[a]	*chiC*	Chitinase
STM1070	*ompA*	Outer membrane porin	PA3479[a]	*rhlA*	Rhamnosyltransferase chain A
STM1572	*ompD*	Outer membrane porin	PA4944	*hfq*	RNA-binding protein Hfq
STM1652	*ynaF*	Putative universal stress protein			
STM2267	*ompC*	Outer membrane porin	*Microaerophilic/anaerobic metabolism*		
STM2638	*rseB*	Anti-αE factor	PA0518	*nirM*	Cytochrome c-551 precursor
STM2640	*rpoE*	SigmaE (α24) factor	PA0519	*nirS*	Nitrite reductase precursor
STM2884	*sipC*	Cell invasion protein	PA0524	*norB*	Nitric-oxide reductase subunit B
STM4361	*hfq*	RNA-binding protein Hfq	PA1557	PA1557	Probable cytochrome oxidase subunit (cbb3-type)
STM4561	*osmY*	Hyperosmotically inducible protein	PA5491	PA5491	Probable cytochrome
Plasmid transfer apparatus			*Hypothetical proteins*		
PSLT081	*traB*	Conjugative transfer	PA0200[a]	PA0200	Hypothetical protein
PSLT095	*traN*	Conjugative transfer	PA1123[a]	PA1123	Hypothetical protein
PSLT099	*trbB*	Conjugative transfer	PA2225	PA2225	Hypothetical protein
PSLT100	*traH*	Conjugative transfer	PA2453	PA2453	Hypothetical protein
PSLT101	*traG*	Conjugative transfer	PA2747	PA2747	Hypothetical protein
PSLT104	*traD*	Conjugative transfer	PA2753[a]	PA2753	Hypothetical protein
PSLT110	*traX*	Conjugative transfer	PA3369	PA3369	Hypothetical protein
SSL_T12	*traT*	Conjugative transfer	PA3520[a]	PA3520	Hypothetical protein
SSL_T20	*traK*	Conjugative transfer	PA3795	PA3795	Probable oxidoreductase
SSL_T24	*traF*	Conjugative transfer	PA4220	PA4220	Hypothetical protein
SSL_T3	*trbC*	Conjugative transfer	PA4351	PA4351	Probable acyltransferase
SSL_T5	*trbA*	Conjugative transfer	PA4352[a]	PA4352	Hypothetical protein
STM_PSLT085	*traR*	Conjugative transfer	PA4633	PA4633	Probable chemotaxis transducer
Small RNAs[b]			PA4739	PA4739	Hypothetical protein
STM_sRNA_α_RBS	*αRBS*	Small RNA			

Table 15.1 (continued)

Salmonella spaceflight			*Pseudomonas* spaceflight		
Gene number	Gene name	Function	Gene number	Gene name	Function
STM_sRNA_CsrB	*csrB*	Small RNA			
STM4124	*oxyS*	Small RNA			
STM_sRNA-RFN	*RFN*	Small RNA			
STM_sRNA_RNaseP	*rnaseP*	Small RNA			
STM_sRNA_rne5	*rne5*	Small RNA			
STM_sRNA_tke1	*Tke1*	Small RNA			
Ribosomal proteins					
STM0469	*rpme2*	50 ribosomal protein L31	PA4433	*rplM*	50 S ribosomal protein L13
STM3425	*rpsF*	30S ribosomal subunit protein S6			
STM3433	*rplP*	50S ribosomal subunit protein L16			
STM3436	*rpsS*	30S ribosomal subunit protein S19			
STM3439	*rplD*	50S ribosomal subunit protein L4			
STM3448	*rpsL*	30S ribosomal subunit protein S12			
STM4150	*rplA*	50S ribosomal subunit protein L1			
STM0469	*rpme2*	50S ribosomal protein L31			
Iron utilization/storage (Fur or Hfq-regulated)					
STM0596	*entE*	2,3–Dihydroxybenzoate–AMP ligase	PA3621	*fdxA*	Ferredoxin I
STM0831	*dps*	Stress response protein and ferritin	PA4880	PA4880	Probable bacterioferritin
STM1660[a]	*fnr*	Transcriptional regulator, Fe binding			
STM1732	*ompW*	Outer membrane protein W			
STM3443	*bfr*	Bacterioferrin, iron storage			

(continued)

Table 15.1 (continued)

Salmonella spaceflight			*Pseudomonas* spaceflight		
Gene number	Gene name	Function	Gene number	Gene name	Function
Biofilm formation					
STM1070	*ompA*	Outer membrane porin			
STM1172	*flgM*	Flagellar biosynthesis			
STM1916	*cheY*	Flagellar biosynthesis			
STM1925	*flhD*	Regulator of flagellar biosynthesis			
STM1962	*fliT*	Flagellar biosynthesis			
Various cellular functions					
–	*mysB*	Suppresses protein export mutants	PA1156	*nrdA*	Ribonucleotide-diphosphate reductase alpha subunit
STM0376	*sbmA*	ABC superfamily transporter	PA1183	*dctA*	C4-dicarboxylate transport protein
STM0536	*ppiB*	Peptidyl-prolyl isomerase B	PA1610	*fabA*	3-hydroxydecanoyl-ACP dehydratase
STM0665	*gltI*	ABC glutamate/aspartate transporter	PA1776	*sigX*	ECF sigma factor SigX
STM0833	*ompX*	Outer membrane protein	PA2003	*bdhA*	3-hydroxybutyrate dehydrogenase
STM0943	*cspD*	Similar to CspA; not cold-induced	PA2247	*bkdA1*	2-oxoisovalerate dehydrogenase (alpha subunit)
STM1066	*rmf*	Ribosome modulation factor	PA2248	*bkdA2*	2-oxoisovalerate dehydrogenase (beta subunit)
STM1290	*gapA*	Glyceraldehyde dehydrogenase	PA2634	PA2634	Isocitrate lyase
STM1682	*tpx*	Thiol peroxidase	PA2851	*efp*	Elongation factor P
STM1749	*adhE*	Fe-dependent dehydrogenase	PA2966	*acpP*	Acyl carrier protein
STM1751	*hns*	DNA-binding protein	PA3686	*adk*	Adenylate kinase
STM1959	fliC	Flagellin, structural protein	PA4031	*ppa*	Inorganic pyrophosphatase
STM2282	*glpQ*	Glycerophosphodiesterase	PA4569	*ispB*	Octaprenyl-diphosphate synthase
STM2488	*nlpB*	Lipoprotein-34	PA5355	*glcD*	Glycolate oxidase subunit GlcD
STM2542	*nifU*	Fe-S cluster formation protein			
STM2665	*yfiA*	Ribosome-associated factor			
STM2801	*ygaC*	Putative cytoplasmic protein			

Table 15.1 (continued)

Salmonella spaceflight			*Pseudomonas* spaceflight		
Gene number	Gene name	Function	Gene number	Gene name	Function
STM2802	*ygaM*	Putative inner membrane protein			
STM3060	*ygfE*	Putative cytoplasmic protein			
STM3285	*rbfA*	Ribosome-binding factor			
STM3648	*yiaG*	Putative transcriptional regulator			
STM3840	*rnpA*	RNase P, protein component			
STM3870	*atpE*	Membrane-bound ATP synthase			
STM3915	*trxA*	Thioredoxin 1, redox factor			
STM4231	*lamB*	Phage λ receptor protein			
STM4392	*priB*	Primosomal replication protein N			

Note: Both up and down-regulated genes with a differential expression value of more than twofold are included and are organized in major functional categories

[a]Also involved in microaerophilic/anaerobic metabolism

[b]Small RNAs were not included in the microarray analysis of *P. aeruginosa*. In addition, the RNA purification kit used for the *S.* Typhimurium microarray analysis had a 200 nucleotide cut-off, therefore additional smaller RNA species may have been inadvertently excluded in this work

differences in cellular aggregation and clumping that was associated with the formation of an extracellular matrix as compared to the ground control cultures (Fig. 15.2d) (Wilson et al. 2007). This observation was consistent with corresponding differences in the expression of genes associated with biofilm formation and may play a role in the enhanced virulence of the organisms grown in space.

15.4 Role of Ion Composition on Spaceflight-induced Virulence

The results of the MICROBE experiment aboard STS-115 led to a follow-up experiment, designated MDRV, aboard STS-123. The goal of this experiment was to (1) confirm the experimental results from STS-115, and (2) determine if altering the ion composition of the growth medium could decrease the spaceflight-induced increase in virulence (Wilson et al. 2008). The hypothesis that manipulation of ion concentrations could counteract or inhibit the spaceflight-associated increase in *Salmonella* virulence was based on initial results from the MICROBE experiment, which suggested that *S.* Typhimurium cultures grown in a minimal carbon, high salt

medium called M9 did not respond with the same spaceflight-induced increase in virulence observed with Lennox broth (LB). In a rare opportunity to replicate a spaceflight result, the data from STS-123 fully supported the results from STS-115 in that (1) *S.* Typhimurium spaceflight cultures grown in LB medium displayed increased virulence in murine infection compared to ground controls, and (2) the spaceflight cultures grown in M9 did not display increased virulence compared to controls (Fig. 15.2). Moreover, the addition of similar concentrations of five key inorganic salts found in M9 medium (NaH_2PO_4, KH_2PO_4, NH_4Cl, NaCl, and $MgSO_4$) to the LB medium reversed the increase in virulence of spaceflight cultures grown in LB medium alone. Interestingly, although different virulence responses were observed in spaceflight cultures grown in the LB and M9 media, significant similarities in gene and protein expression profiles indicated involvement of the Hfq regulon in either media. Subsequent ground-based investigations using the RWV reinforced the flight data indicating an inhibitory effect of high ion concentrations in the growth medium on pathogenesis-related responses. By systematically adding different combinations of the inorganic salts to the LB medium, phosphate ion (PO_4) was isolated as the key component to repressing this microbial pathogenesis-related response (Wilson et al. 2008).

New discoveries using *S.* Typhimurium as a model organism are continuing as a follow-up flight experiment to build upon results obtained from MICROBE and MDRV, and were flown on STS-131 (April, 2010). This experiment, designated as STL-IMMUNE, was the first experiment to conduct an in-flight infection of human cells (intestinal) with a microbial pathogen (*S.* Typhimurium). The data from this spaceflight experiment will provide insight into alterations in host–pathogen interactions that occur during spaceflight and will unveil the cellular and molecular mechanisms behind those changes. This information has the potential to significantly change our microbial risk assessment and operational requirements during a mission.

15.5 Studying the Response of *Pseudomonas Aeruginosa* to Spaceflight and Spaceflight Analogue Conditions Reveals Similarities and Differences As Compared to the *Salmonella* Response

As a versatile, ubiquitous bacterium that is occasionally part of the normal human flora, *P. aeruginosa* can also survive in extraterrestrial habitats, as evidenced by its isolation from the potable water system on the ISS and from Apollo crewmembers (Taylor 1974; Hawkins and Ziegelschmid 1975; Castro et al. 2004; Bruce et al. 2005). Astronaut cross-contamination with *P. aeruginosa* has been reported during short-term missions emphasizing the potential of this infectious agent to rapidly spread among crewmembers (Taylor 1974). Thus far, the presence of *P. aeruginosa* in the spaceflight vehicle has led to one reported severe urinary tract infection in-flight (Taylor 1974). In addition to the importance for astronaut safety, studying

the behavior of *P. aeruginosa* in the low fluid-shear conditions of microgravity provides insights into the role that low fluid-shear regions in the human body play in triggering virulence characteristics.

The transcriptional and proteomic responses of *P. aeruginosa* to spaceflight conditions were profiled as a part of the MICROBE experiment (Crabbé et al. 2011). Intriguingly, Hfq and a significant part of the Hfq regulon were differentially regulated by *P. aeruginosa* in spaceflight. As described above, Hfq was also identified as a key regulator in the LSMMG and spaceflight response of *S.* Typhimurium (Wilson et al. 2002b, 2007, 2008). Hence, Hfq is the first transcriptional regulator ever shown to be involved in the spaceflight response across the bacterial species border. Among the genes with the highest fold inductions in spaceflight-grown *P. aeruginosa* were the genes encoding the lectins, LecA and LecB. Lectins play a role in the bacterial adhesion process to eukaryotic cells, and have clinically important cytotoxic effects (Gilboa-Garber et al. 1977; Bajolet-Laudinat et al. 1994; Chemani et al. 2009). Another virulence gene that was induced by *P. aeruginosa* in response to spaceflight culture conditions was *rhlA*, which encodes the rhamnosyltransferase I involved in rhamnolipid surfactant biosynthesis. Rhamnolipids are glycolipidic surface-active molecules that have cytotoxic and immunomodulatory effects in eukaryotic cells (McClure and Schiller 1996; Davey et al. 2003; Pamp and Tolker-Nielsen 2007). Furthermore, spaceflight induced genes and proteins involved in the anaerobic growth of *P. aeruginosa*. Indeed, more limited oxygen availability could occur in spaceflight conditions due to low fluid-shear and thus, low mixing growth conditions. Future studies will determine if the phenotype of *P. aeruginosa* acquired under spaceflight conditions could effectively lead to increased pathogenicity, as was observed for *S.* Typhimurium.

The cultivation of *P. aeruginosa* PAO1 in spaceflight analogue conditions (LSMMG) in the RWV bioreactor at 28°C induced a transcriptomic and phenotypic profile related to virulence (Crabbé et al. 2010). More specifically, *P. aeruginosa* PAO1 produced higher amounts of the exopolysaccharide alginate when grown in LSMMG, as compared to the control. Alginate is an important virulence factor in *P. aeruginosa* since it restricts the diffusion of anti-microbial agents and confers resistance to immune defense mechanisms, by avoiding phagocytic uptake, scavenging reactive oxygen species and suppressing leukocyte function (Learn et al. 1987; Pier et al. 2001). Accordingly, an increased oxidative stress resistance in LSMMG-grown *P. aeruginosa* was observed, as well as a higher transcription of the alternative sigma factor *algU*, essential for alginate production. Interestingly, Hfq was also found to be an important regulator in the LSMMG response of *P. aeruginosa* (Table 15.1). This transcriptional regulator is thus key in the spaceflight and LSMMG responses of both *S.* Typhimurium and *P. aeruginosa* (Wilson et al. 2002b, 2007, 2008; Crabbé et al. 2010, 2011). In addition, the *P. aeruginosa* LSMMG regulon (comprised of 134 genes) included genes involved in stress resistance, motility, and microaerophilic/anaerobic metabolism. As mentioned above, oxygen limitation could be inherently linked to growth in lower fluid-shear (both in LSMMG and in actual microgravity).

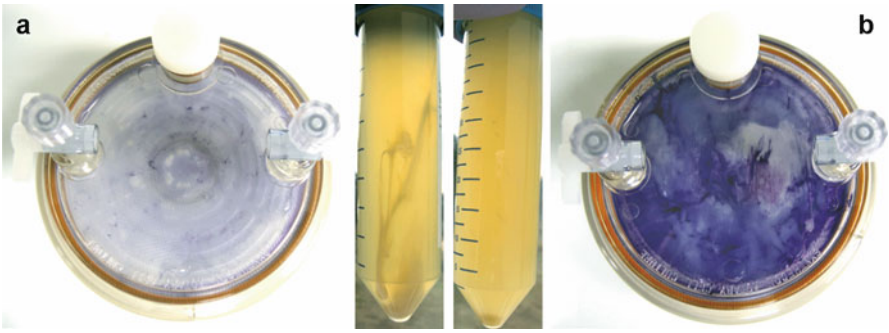

Fig. 15.3 *Biofilm formation of P. aeruginosa PAO1 in low shear modeled microgravity (LSMMG) (***a***) and higher shear (***b***) conditions using the RWV technology.* The gas-permeable membrane on the backside of the vessel was stained with Crystal Violet. The decanted culture was collected in 50 ml falcon tubes (center)

Another impact of low fluid-shear was demonstrated in a study where LSMMG-grown *P. aeruginosa* (37°C) formed dense self-aggregating biofilms (Fig. 15.3a) (Crabbé et al. 2008). In contrast, a membrane-attached biofilm was formed in high fluid-shear, while no self-aggregation could be observed (Fig. 15.3b). Interestingly, the phenotypic and transcriptomic profiles of *P. aeruginosa* grown in LSMMG showed similarities with that of *P. aeruginosa* found in lung secretions of cystic fibrosis (CF) patients (Lam 1980; Singh et al. 2000; Sriramulu et al. 2005; Bjarnsholt et al. 2009). In CF patients, the formation of ultraresistant microcolonies by *P. aeruginosa* in the dense and viscous lung mucus is the major cause of mortality (Wagner and Iglewski 2008). Low fluid-shear zones are believed to be present in the lung mucus of CF patients due to the absence of mucociliary clearance, which represents the main shear-causing factor in the normal lung mucus (Blake 1973). Hence, this study demonstrated for the first time that low fluid-shear could potentially be one of the factors contributing to the development of the specific biofilm phenotype of *P. aeruginosa* in CF lungs.

15.6 How Universal Is the Microbial Response to Spaceflight and Spaceflight Analogue Culture?

The studies described in the previous sections involving *S.* Typhimurium and *P. aeruginosa* provide key examples of two biomedically important human pathogens that exhibit a variety of similarities and differences in their responses to spaceflight and spaceflight-analogue culture. While these organisms have received the most extensive degree of study, the response of other pathogens to these environments have also been investigated, and results from these studies suggest both potential common mechanisms as well as a myriad of different responses.

For example, *Staphylococcus aureus* is a Gram-positive bacterium of particular importance to crew health due to its prevalence and reported transmission aboard spacecraft (Pierson et al. 1996; Castro et al. 2004). Interestingly, RWV-cultured *S. aureus* displayed slower growth and generally repressed virulence characteristics, including decreased carotenoid production (Rosado et al. 2010; Castro et al. 2011), decreased capacity to lyse red blood cells (Rosado et al. 2010), increased susceptibility to oxidative stress (Castro et al. 2011), reduced survival in whole blood (Castro et al. 2011), and intriguingly, increased formation of a biofilm phenotype (Castro et al. 2011). Furthermore, molecular genetic expression analysis revealed the down-regulation of the RNA chaperone protein Hfq, which parallels the response of *S.* Typhimurium to LSMMG culture. This common association with Hfq in both Gram-positive and Gram-negative organisms suggests an evolutionarily conserved response to fluid-shear among structurally diverse prokaryotes (Castro et al. 2011). However, unlike *S.* Typhimurium and *P. aeruginosa*, these results suggest *S. aureus* responds to the RWV environment by initiating a biofilm phenotype with diminished virulence characteristics. Collectively, these comparisons may provide novel insight into key factors influencing the delicate balance between infection and colonization by *S. aureus* during the initial host–pathogen interaction.

The number and variety of microorganisms that have been studied in spaceflight and spaceflight analogue culture is extensive, and beyond the scope of this chapter. However, examples of key findings from experiments investigating the responses of medically significant microorganisms during culture in these environments are presented in Table 15.2.

15.7 Importance to Spaceflight and Life on Earth

As humans explore space, microorganisms will travel with them. Thus, understanding microbial responses to spaceflight will have a tremendous impact on how we design our spaceflight vehicles, build bioregenerative systems, grow food during a mission, and mitigate the risk of infectious disease to the crew. While several key studies have provided critical mechanistic insight into how microbial responses to the spaceflight environment may affect virulence, many questions still remain unanswered. Indeed, spaceflight-induced alterations in microbial virulence have just begun to be investigated. The impact of these changes on the host–pathogen interaction during spaceflight and corresponding clinical implications for a potentially susceptible crew is still unclear. Beyond prevention of exposure, antibiotics are the primary countermeasure to microbial infection during a spaceflight mission. Previous spaceflight experiments have identified potential increases in antibiotic resistance for organisms such as *E. coli* (kanamycin and colistin) and *S. aureus* (oxacillin, chloramphenicol, and erythromycin) in response to spaceflight culture (Tixador et al. 1985). The lack of conclusive information on changes in antibiotic resistance of a broad range of microorganisms and corresponding pharmacokinetics

Table 15.2 Key findings from experiments investigating medically significant microorganisms cultured in spaceflight and spaceflight analogue conditions

Microorganism	Environment	Finding
Escherichia coli	LSMMG	Increased resistance to a variety of pathogenesis-related stresses, including low pH, osmotic, alcohol, and thermal stress (Gao et al. 2001; Lynch et al. 2004, 2006; Allen et al. 2008)
	LSMMG	Acid and osmotic stress resistance was shown to be RpoS-independent in the exponential phase of culture (similar to the findings for *S.* Typhimurium cultured to exponential phase in the RWV (Wilson et al. 2002a)), but RpoS-dependent in stationary phase (Lynch et al. 2004)
	LSMMG	Dense biofilms that exhibited increased resistance to some environmental stresses and antibiotics were observed when *E. coli* was cultured to stationary phase under LSMMG conditions, as compared to control conditions, in the presence of glass microcarrier beads (Lynch et al. 2006)
Enterotoxigenic *E. coli* (ETEC)	LSMMG	Carvalho et al. (2005) infected a 3-D model of colonic epithelium with either LSMMG-cultured EPEC or EHEC, respectively, when both the host and pathogen were simultaneously cultured in the RWV bioreactor. Formation of attaching and effacing lesions similar to that observed during the normal course of infection *in vivo*, and increased intimin expression (an outer membrane protein mediating adherence to intestinal epithelium) were observed during EHEC infection of 3-D colon cells in the RWV
Enteropathogenic *E. coli* (EPEC)	LSMMG	Increased toxin production was observed in ETEC cultured under LSMMG conditions as compared to control cultures, which correlated with increased fluid accumulation in mice infected with LSMMG-cultured ETEC (Chopra et al. 2006). Moreover, an increase in TNF-α production was observed in macrophages infected with EPEC cultured under LSMMG conditions as compared to macrophages infected with control cultures
Adherent-Invasive *E. coli*	LSMMG	Displayed increased adherence to $Caco_2$ colonic epithelial cells when the bacteria were cultured under LSMMG conditions; an effect which became even more pronounced when the same study was performed with an *rpoS* mutant (Allen et al. 2008)
Streptococcus pneumoniae	LSMMG	Demonstrated differential transcriptional regulation of 101 genes, including those involved in the adherence and invasion of *S. pneumoniae* (Allen et al. 2007)

Table 15.2 (continued)

Microorganism	Environment	Finding
Saccharomyces cerevisiae	Apollo 16	Increased phosphate uptake was observed during spaceflight culture (Berry and Volz 1979)
	Apollo 16	Increased survival rates in tissues following multiple infection routes in a mouse model of infection (intraperitoneal, tail vein, and epidermal) as compared to ground-based controls (Hiebel and Volz 1977; Volz 1990)
	LSMMG	In RWV studies with *S. cerevisiae*, a random budding pattern and tendency to self-aggregate and clump has been found to occur in response to LSMMG culture in contrast to the normal bipolar budding phenotype normally observed in controls. These differences were accompanied by marked changes in the expression of genes important for polarity, budding, and cell separation (Purevdorj-Gage et al. 2006)
	LSMMG	Microarray analysis revealed that a large percentage of the *S. cerevisiae* genome responded to LSMMG culture, and that many of the genes found to be differentially regulated are known to be important for general environmental stress responses (Johanson et al. 2002; Sheehan et al. 2007)
Candida albicans	LSMMG	Random budding pattern and increased clumping as compared to control cultures An increased frequency of filamentous forms in LSMMG cultures was accompanied by significant changes in the expression of two genes, *hwp1* and *ywp1*, which are associated with yeast-hyphal transition. The morphogenic switch from round, budding yeast to filamentous form is often associated with enhanced virulence of the microorganism (Altenburg et al. 2008)

indicates a large knowledge gap in infectious disease control. Moreover, understanding the microbiota to which the crew will be exposed, and how spaceflight alters the microbial consortia and interactions with the crew, is one of the cornerstones of microbiological risk assessment during a mission. While microorganisms associated with the environment and food supply have been the focus of operational monitoring efforts, very little is known about the changes in crew microbiome during a mission. Likewise, little is known about mutation rates and heritable changes in all microorganisms associated with the crew and their environment during a mission – and nothing is known of long-term spaceflight-induced changes to either the host or pathogen. Alone or in combination, these factors could dramatically affect the impact of microorganisms on spaceflight mission success. As we

look to the future and introduction of commercial spaceflights with greater civilian participation, including spaceflight tourism, we need a greater understanding of the unique microbial risks associated with human spaceflight. Moreover, lessons learned from spaceflight studies have profound implications for the general public, in terms of expanding our knowledge of (1) the mechanisms of microbial pathogenesis, which hold potential for development of novel strategies to diagnose, treat, and prevent infectious disease, and (2) the human microbiome and how stressful environments alter the relationship between host and commensal that determine the transition between normal homeostasis and disease progression (http://commonfund.nih.gov/hmp/).

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Stress, Spaceflight, and Latent Herpes Virus Reactivation

16

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16.1 Stress Responses Associated with Spaceflight

Stress responses are controlled through a complex network of neural, humoral, and metabolic factors among which the hypothalamic–pituitary–adrenocortical (HPA) axis and the sympathetic–adrenal–medullary (SAM) axis play a crucial effector role (see also Chaps. 3 and 4). In brief, while activation of the HPA axis is thought to occur during events perceived as more overwhelming and less readily coped with, activation of the SAM axis is associated with stressful emotions such as anger and fear. The neuroendocrine response is extremely complex and involves numerous feedback mechanisms. Individuals may manifest different levels of stress hormones due to variables such as prior exposure to the stressor, behavior, and coping responses. Due to sampling and stowage constraints during spaceflight, most of the data on stress hormone changes have been derived from samples taken after landing and comparing them to samples taken before launch.

Even before spaceflight, neuroendocrine changes can occur. An increase in serum cortisol was found in two cosmonauts 15 days prior to launch as compared to 2 months before (Caillot-Augusseau et al. 1998). Plasma cortisol was also elevated in several Shuttle astronauts 10 days prior to launch as compared to their annual medical exams, a period well removed from launch and thought to be a period of low stress (Stowe et al. 2000). More recent studies have confirmed that astronauts

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experience considerable stress just prior to launch (Mehta et al. 2000, 2001; Pierson et al. 2005). Thus, timing of biological sample collection relative to launch must be considered during the experimental design phase to account for preflight stress.

Although in-flight data are very limited, a pattern has emerged during the early phase of flight. A significant elevation in urinary cortisol occurred immediately after launch in one mission specialist (Leach 1987). In the Spacelab Life Sciences (SLS) missions, urinary cortisol was also significantly increased immediately after launch in both crews (Leach et al. 1996). Cortisol returned to preflight levels on flight days 2–9 of SLS-1, whereas in SLS-2 urinary cortisol was significantly elevated throughout most of the flight. In the STS-95 mission, plasma cortisol was significantly elevated after launch in one crewmember and remained elevated for the first four flight days (Stowe et al. 2001a). Catecholamines generally are not elevated during Shuttle spaceflights indicating little or no sympathetic adrenal activation (Smith et al. 1997), but as previously stated landing stresses result in significant elevations in plasma and urinary catecholamines.

In the later, "adaptive" phase of spaceflight, cortisol has been reported to either not change or increase. During Skylab, plasma and urinary cortisol were generally increased throughout the flight (Leach and Rambaut 1977). Plasma cortisol was increased on days 216–219 of the 237-day Salyut flight; no change was observed in urinary cortisol (Gazenko et al. 1988). Urinary cortisol was reduced on flight days 43–45 but increased on flight day 88 during the Salyut-7 (Vorobyev et al. 1986). Plasma cortisol was unchanged from preflight levels toward the end of a 241-day Mir mission (Grigoriev et al. 1990). During Mir, samples obtained from two subjects on flight days 88–186 showed an increase in urinary cortisol while four others showed a decrease (Stein et al. 1999). It has been proposed that the variability in cortisol levels during long-duration missions may be due to mission-specific stress (Stein 1999), changes in the steroidogenesis pathway (Vorobyev et al. 1986), disruption of circadian rhythms (Leach-Huntoon et al. 1996), and negative feedback loops (Leach et al. 1996).

In summary, multiple factors associated with spaceflight (e.g., hypergravity, microgravity, confinement, separation from family, sleep deprivation) elevate stress hormones and reflect activation of the HPA axis. There is increasing evidence that spaceflight and associated stressors result in a shift toward Th2 cytokine profile (see Chap. 12). Glucocorticoids are primarily anti-inflammatory in that they inhibit IL-12 production and increase IL-10 production by monocytes which drives the immune response toward a Th2 cytokine profile (IL-4, IL-5, IL-10) and away from a Th1 profile (IL-2, IL-12, IFN-γ) (Elenkov et al. 1996). Decreased production of Th-1 cytokines has been documented repeatedly in astronauts (reviewed in Taylor et al. 1997); these findings are underscored by the inhibited delayed-type hypersensitivity response observed during short-term (Taylor 1993) and long-term missions (Konstantinova et al. 1993). The hypothesis is also supported by data from Stein et al. (1997), who showed that IL-10 levels spike immediately after launch and correlate with increased levels of cortisol. Moreover, glucocorticoids enhance IL-4 and IL-10 synthesis resulting in increased circulating IgE levels in vivo (Zieg et al. 1994; Ramierz et al. 1996), and we have recently noted increased IgE levels after short-term spaceflights (Stowe et al. 2001a, 2003). Accordingly, it has been proposed that a Th1-to-Th2 shift may increase susceptibility to opportunistic infections such as viral pathogens (Elenkov et al. 1996).

16.2 Reactivation of Latent Herpes Viruses in Astronauts

Although infectious disease risks associated with most pathogens (i.e., those that cause acute infections) may be reduced by a quarantine period before spaceflight, latent viruses are not mitigated by a quarantine period and are subject to intermittent reactivation. Herpesviruses, the best known latent viruses, commonly establish infections in humans. The family of herpesviridae, which includes herpes simplex type-1 and type-2 (HSV-1, -2), cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella-zoster virus (VZV), human herpesvirus-6, -7, and -8 (HHV-6, -7, -8) are all double-stranded DNA viruses that may establish a lifelong latent infection in the host. These infections are characterized by an acute phase usually associated with minor morbidity and mortality: productive viral replication occurs in a sequential manner consisting of immediate-early gene expression, transcriptional transactivation, early gene expression, viral genome replication, late gene expression, nucleocapsid assembly, viral genome packaging, and release of enveloped virions from the cell. Primary infection is followed by a chronic latent phase reflecting a balance between viral replication and the host immune response.

Herpes viruses are medically important viruses; HSV-1 infects 70–80% of all adults and is classically associated with oropharyngeal lesions such as cold sores, pharyngitis, and tonsillitis. EBV infects over 85% of the adult population and is the causative agent of infectious mononucleosis, Burkitt's lymphoma, undifferentiated nasopharyngeal carcinoma, and diffuse polyclonal B-cell lymphoma. Most CMV infections in adults are asymptomatic but may result in an infectious mononucleosis-like syndrome, central nervous system infections, and febrile illnesses. Notably, CMV infections can be severe in immunocompromised individuals such as AIDS and post transplant patients (Komanduri et al. 2001). VZV causes chicken pox on primary infection and remains latent thereafter; VZV may reactivate resulting in episodes of zoster or "shingles" (Arvin 1996).

Herpes virus reactivation appears to be triggered by stress (Fig. 16.1). Prior ground-based studies of chronic stress have demonstrated that reduction or loss of cellular immune function results in latent herpes viruses. Glaser and colleagues have demonstrated decreased cellular immunity and increased antibodies to EBV in chronically stressed individuals (Glaser et al. 1985, 1991, 1993) (see also Chap. 4). Although virus reactivation in these individuals was not associated with clinical disease, other studies have linked psychological stress with onset and severity of infectious mononucleosis (Kasl et al. 1979). Moreover, significant immunosuppression (i.e., organ transplant recipients) correlates directly with the development of EBV-related lymphoproliferative diseases (Preiksaitis et al. 1992; Rea et al. 1994; Haque and Crawford 1997). Given the alterations in the immune system during spaceflight, a major concern is the development of an EBV-associated disease or lymphoma during long-term spaceflight. In addition, reinfection or transmission to a previously uninfected individual (resulting in primary infection) may be another concern.

We have been investigating reactivation of latent herpes viruses in astronauts by measuring anti-viral antibodies and shedding of viral DNA, most notably to EBV, CMV, and VZV. The results of our studies and interpretation of the results are discussed.

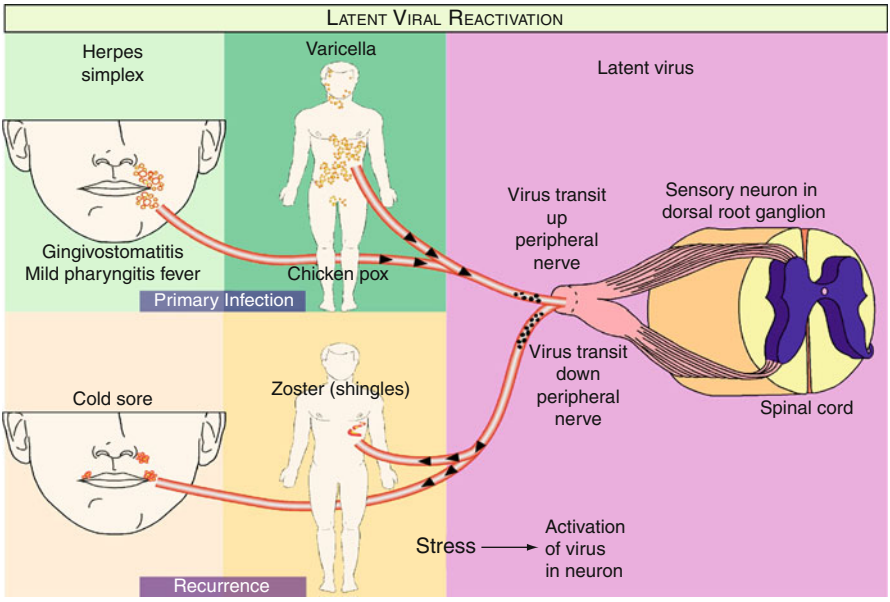

Fig. 16.1 Primary infection and reactivation of herpesvirus

16.2.1 Epstein-Barr Virus

EBV infects approximately 90% of the adult population and is closely associated with malignancies such as Burkitt's lymphoma and nasopharyngeal carcinoma (Cohen 2000). Following primary infection, EBV remains latent in healthy individuals and undergoes occasional reactivation. In previously infected (seropositive) individuals, anti-viral capsid antigen (VCA) and anti-EBV nuclear antigen (EBNA) IgG antibodies are always present. A dysfunction of the immune surveillance system against EBV infection permits productive cycles of viral replication, which lead to increased production of early antigen (EA) and VCA IgG antibodies. Increased viral shedding in saliva can also be detected.

In our initial study on latent virus reactivation, saliva samples were collected from 11 EBV-seropositive astronauts before, during, and after spaceflight (Payne et al. 1999). EBV DNA was detected more frequently before space shuttle missions than during ($P<0.001$) or after ($P<0.01$) missions; no significant difference in frequency was detected between the in-flight and postflight periods (Payne et al. 1999). In addition to viral DNA, the titers of antibodies to EBV antigens VCA and EA were measured in blood samples taken from astronauts at their annual medical exams (baseline), 10 days before launch (L – 10), a few hours after landing (R + 0), and 3 days after landing (R + 3). The titer of anti-VCA antibodies was greater at L-10 than the baseline value (Stowe et al. 2000, 2001a, b; Pierson et al. 2005) indicating increased viral replication. Notably, the titer of anti-Epstein-Barr virus nuclear antigen (EBNA) antibodies (which positively correlates with T-cell function)

was less than its titer at the baseline reflecting decreased cellular immunity (Stowe et al. 2001b). In a subsequent study that used quantitative real-time PCR, The mean EBV DNA copy number was significantly greater during flight than either preflight or postflight (Pierson et al. 2005). We also found that the amount of EBV DNA shed in the saliva of astronauts during spaceflight increased as the number of days in space increased. Similar data from two cosmonauts aboard the Russian space station Mir showed that EBV shedding in saliva occurred throughout the nearly 3-month mission, a much longer time than the relatively short (<14 days) duration of the shuttle missions. However, the number of EBV copies shed by cosmonauts aboard Mir did not increase as a function of days in flight, as observed with astronauts on the space shuttle. The maximum number of EBV copies shed by cosmonauts on Mir was 1130/mL of saliva, and the maximum number of copies shed by astronauts on the shorter shuttle flights was 738/mL of saliva.

16.2.2 Cytomegalovirus

Another latent herpesvirus, human CMV, may pose a similar kind of risk to astronauts' health during spaceflight. CMV infection is typically acquired asymptomatically during childhood. However, in individuals whose immune system is either immature or immunocompromised (e.g., HIV infection), CMV can cause multiple diseases such as encephalitis, gastroenteritis, pneumonia, and chorioretinitis (Fiala et al. 1975). Moreover, several studies have suggested that CMV infection may contribute to preexisting immunosuppression by directly infecting leukocytes as well as hematopoietic cells (Carney and Hirsch 1981; Rice et al. 1984; Simmons et al. 1990). We therefore examined the effects of spaceflight on CMV reactivation and shedding.

In our first study of CMV in astronauts, CMV reactivation and shedding was examined in urine samples collected from 71 crew members from short-term space missions (Mehta et al. 2000). The frequency of CMV DNA shedding in either pre- or postflight urine samples from astronauts was significantly higher than that of the control population ($P < 0.05$). CMV DNA was detected in 27% of the crew members studied during the mission monitoring period. By contrast, only 1 of the 61 control subjects shed CMV during one sampling period. We had an opportunity to sample both blood and urine from two astronauts during a subsequent flight. Consistent with the earlier study (Mehta et al. 2000), CMV was shed in urine of one crew member before, during, and after flight, and in urine of the other crew member only during flight (on 2 days) (Stowe et al. 2001a).

Of the 71 crew members, 55 (77%) were seropositive. As a group, no significant changes in anti-CMV antibodies were found postflight. However, upon dividing these subjects between 40 nonshedders and 15 CMV shedders, an interesting difference was found. No significant change in CMV IgG antibody titer of nonshedders was found at any time point compared to the baseline values. In contrast, the anti-CMV antibody titer of the 15 shedders was significantly increased at all time points compared to their baseline values. Furthermore, anti-CMV antibodies

was significantly increased at R + 3 compared to L–10. The anti-CMV antibody titers of the control subjects did not differ significantly from the baseline levels of astronauts, and no changes in anti-CMV IgG antibody titer from 11 CMV-seropositive control subjects were found across three sampling times (Mehta et al. 2000). Overall the results of our CMV studies demonstrated that CMV reactivation occurred in astronauts and may pose another health problem during longer-duration missions.

16.2.3 Varicella Zoster Virus

VZV is a highly successful human pathogen. Primary VZV infection typically results in childhood varicella (chickenpox). Varicella is characterized by malaise, fever, and an extensive vesicular rash. Varicella is normally self-limiting and resolves with the development of humoral and cell-mediated immunity. VZV is transmitted by direct contact with vesicle fluid or aerosolized respiratory tract secretions (Kavaliotis et al. 1998). VZV DNA is present in the air in hospital rooms of varicella or zoster patients (Sawyer et al. 1994). While the air and even remote surfaces around zoster patients can contain VZV DNA (Yoshikawa et al. 2001), only recently has infectious virus been recovered in saliva from a zoster patient (Mehta et al. 2008). VZV is highly contagious with a secondary attack rate approaching 100% (Asano et al. 1977) that can lead to epidemics in places of close confinement.

While widespread aggressive vaccination has greatly lessened the morbidity and mortality associated with varicella (Arvin and Gershon 1996), in countries where vaccination is not mandatory, deaths still occur (Rawson et al. 2001).VZV reactivation, predominately in the elderly, most often results in zoster (shingles), but virus may spread to the spinal cord to cause myelitis or to blood vessels of the brain to cause vasculopathy. VZV reactivation often produces prolonged pain after zoster (postherpetic neuralgia).

While the clinical features of VZV reactivation are well recognized, subclinical VZV reactivation and shedding has recently been reported in astronauts. Physical and physiological stressors associated with spaceflight (Taylor and Janney 1992; White and Averner 2001; Williams 2003) appear to induce virus reactivation and subsequent shedding of VZV in saliva (Mehta et al. 2004; Cohrs et al. 2008). Herein, asymptomatic VZV reactivation during spaceflight, in ground-based spaceflight analogs, and in the astronauts before, during, and after short-duration spaceflight is reviewed.

16.2.3.1 Asymptomatic VZV Reactivation and Virus Transmission

Prolonged maintenance of anti-VZV antibodies in small, isolated populations supports the notion of subclinical reactivation; however this finding equally supports periodic boost in anti-VZV titer due to subclinical reactivation of latent VZV (Hope-Simpson 1965; Black et al. 1974). The eyes are a likely site to demonstrate subclinical VZV reactivation since, both HSV-1 and VZV become latent in trigeminal ganglia (Cohrs et al. 2005) and even within the same neuron (Theil et al. 2003), both

viruses cause keratitis following reactivation (Reijo et al. 1983; Cook et al. 1986; Kaye et al. 2000). However, using equally sensitive PCR technology, VZV DNA was not detected in tear film from 35 normal subjects (Yamamoto et al. 1994; Willoughby et al. 2002) or conjunctival scrapings from 30 individuals with eye trauma or unrelated disease (Lee-Wing et al. 1999). Thus, unlike HSV-1, where asymptomatic virus reactivates often and can be detected frequently in tear film, asymptomatic VZV reactivation is either a rare event or does not progress to yield virus in the eyes.

In an effort to understand the prevalence of virus reactivation in the unique environment of space, DNA extracted from 312 saliva samples were collected from eight astronauts (two short-term shuttle missions). VZV DNA was detected in only 1 of 112 saliva samples taken 234–265 days before flight, whereas during and shortly after spaceflight 61 of 200 saliva samples were positive for the virus DNA (Mehta et al. 2004). No VZV DNA was detected in saliva from 10 age-matched control subjects (88 samples). Subsequently, VZV DNA was again detected in saliva samples from 2 of 3 space shuttle astronauts during and within days following spaceflight, but not before launch (Cohrs et al. 2008). Importantly, infectious VZV was isolated in saliva samples following landing from the two astronauts whose saliva contained VZV DNA. These results suggest asymptomatic VZV reactivation does occur, and saliva is a convenient source material for its detection. While it is impossible to determine if the virus in both astronauts resulted from simultaneous reactivation, or if a single reactivation and shed in one subject resulted in asymptomatic infection of the second subject, the presence of a rare restriction endonuclease recognition site in the VZV DNA isolated from both subjects argues for the latter case.

A Phase 2 trial was conducted to evaluate the efficacy of a topical antiviral, sorivudine, in treatment of acute herpes zoster as an adjuvant to valacyclovir. In this randomized, placebo-controlled, double-blind trial, 25 patients were treated with either sorivudine or placebo cream. VZV DNA was detected in serum, zoster lesion swabs, and peripheral blood mononuclear cell (PBMC) samples. The number of viral copies correlated with progression of herpes zoster. The detection of VZV DNA in the serum and PBMCs of all 25 zoster patients documents that viremia is a common manifestation of herpes zoster (Satyaprakash et al. 2009).

The PCR assay is specific, sensitive, and rapid (same day results), but a skilled operator and an expensive equipment are required. We developed a new kit for physicians that can detect the presence of VZV DNA in saliva (or urine) in ~15–20 min with no additional equipment needed (Provisional Patent Application). This technology is especially useful for an individual with dermatomal pain of unknown origin. The primary application for this technology is for early diagnosis of shingles patients. Typically, individuals destined to develop shingles experience pain in a dermatomal region with no apparent cause. If the pain is due to the onset of shingles, early diagnosis with this technology allows early intervention with prevention or blunting of the zoster rash and less nerve damage. Early treatment will likely reduce the number of postherpetic neuralgia patients. This study demonstrated the practical application of NASA-developed technology to everyday life.

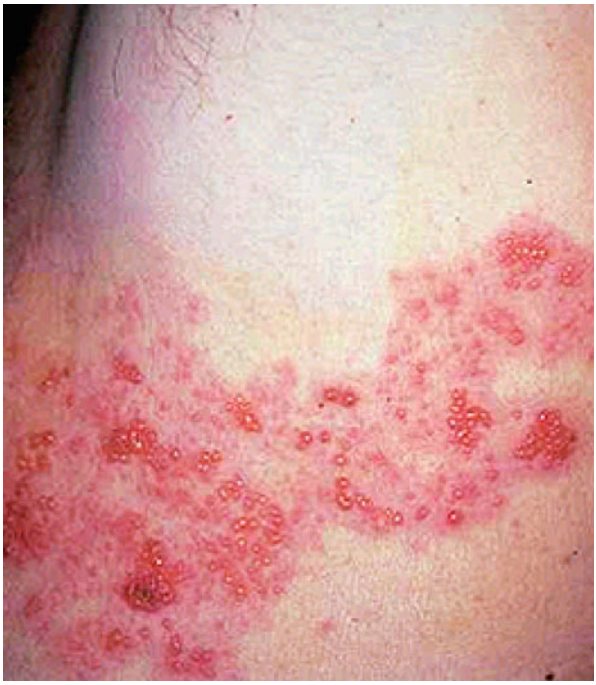

Fig. 16.2 The characteristic painful rash of shingles

16.2.3.2 Clinical Importance of Asymptomatic VZV Shedding

The most obvious sign of VZV reactivation is vesicular rash and pain associated with zoster (Fig. 16.2), however in the absence of rash, the virus can also spread to the retina causing blindness, to the spinal cord causing paralysis and incontinence, and to cerebral arteries resulting in stroke (Kleinschmidt-DeMasters and Gilden 2001; Orme et al. 2007). While associating VZV with disease is straightforward when rash and vesicles are present, correlating VZV with disease is difficult when the outward signs are absent. For example, when stroke occurs in the elderly, especially many months following zoster, the association with VZV reactivation requires cerebral spinal fluid analysis for VZV antibodies (Nagel et al. 2007). Likewise, detection of asymptomatic VZV reactivation which often is only seen as an increase in antibody titer against VZV (but may also result in virus transmission) is difficult to detect.

16.3 Summary

Spaceflight represents a unique environment that is comprised of multiple stresses before, during, and after flight and result in activation of the HPA and SAM axes and subsequent increased circulating concentrations of cortisol, catecholamines, and neuropeptides. Many immune functions are consistently diminished in astronauts immediately after spaceflight, and a spaceflight–induced shift to a Type 2

cytokine pattern is becoming evident. Alterations in immunity and stress hormone levels result in reactivation of latent herpes viruses. The medical significance of altered immunity and increased, but asymptomatic, viral reactivation in astronauts is unknown, but it is possible that spaceflight–induced changes in immunity may increase during the exploration missions, some of which will have much longer durations than current International Space Station missions. Further decreases in immunity may result in greater risks of active infection by opportunistic pathogens, including latent herpes viruses. These studies of asymptomatic reactivation of latent herpes viruses in an extremely healthy group of subjects may provide new insight into stress, immunity, and viral disease in the general population.

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Stress and Radiation Responsiveness

17

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Filip Vanhavere, and Sarah Baatout

17.1 The Space Radiation Environment

17.1.1 Definition and Source of Cosmic Rays

A cosmic ray is a high-speed particle, either an atomic nucleus or an electron that travels throughout the entire Milky Way Galaxy. The term *ray* is a misnomer, as cosmic particles arrive individually, not in the form of a ray or a beam of particles.

Some of these particles originate from the Sun, but most come from sources outside the solar system perhaps even from outside the galaxy and are known as *galactic cosmic rays* (GCRs). The main source of GCR is believed to be supernovae (also known as exploding stars), while occasionally a disturbance in the sun's atmosphere (such as a solar flare or a coronal mass ejection, also known as solar particle event (SPE)), leads to a surge of radiation particles with sufficient energy to penetrate the earth's magnetic field and to enter the atmosphere. SPE occurs intermittently and, as yet, unpredictably. Almost all solar cosmic rays have enough energy to ionize the various gases in the upper atmosphere. However, most of the CGRs that finally reach the Earth's surface are shielded by the atmosphere. Some cosmic rays can be trapped in the magnetic field of the galaxy and can therefore approach the spacecraft from all directions. The aurora borealis and australis are wonderful illustrations of such ionization on earth. Noteworthy is that large solar flares produce sufficient solar wind to cause radio interference and sometimes even damage to satellites.

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17.1.2 Primary and Secondary Cosmic Rays

There are two categories of cosmic rays: *primary cosmic rays* (also called primaries) and *secondary cosmic rays*. Technically, primary cosmic rays are those particles accelerated at astrophysical sources while secondary cosmic rays are resulting from the interaction of the primaries with interstellar gas, atmosphere, or any atom (such as those comprising the space vehicle shielding) encountered. Electrons, protons, and helium, as well as carbon, oxygen, iron, and other nuclei synthesized in stars, are primaries. Secondary cosmic radiations originate when primary cosmic rays interact with atmospheric atoms and give rise to cosmogenic radionuclides which induce radioactivity in the environmental media.

Primary cosmic radiation is composed of 98% atomic nuclei of which protons make up 86%, helium ions 11%, and a small but important 1% consists of heavier ions called High Z and High-Energy (HZE) particles (so named for their high atomic number (Z) and energy (E)). Iron is the most important HZE because of its high-linear energy transfer (LET, a measure of the energy transferred to material as an ionizing particle travels through it) and its contribution to the GCR dose. To note, shielding has little effect on HZE particles. The remaining 2% of the primary cosmic radiation consist of electrons.

Nuclei such as lithium, beryllium, and boron (which are not abundant end-products of stellar nucleosynthesis, the nuclear reactions taking place in stars to build the nuclei of the elements heavier than hydrogen) are typical secondaries. Cosmogenic radionuclides can include 3H, 7Be, 10Be, 14C, 27Na, 26Al, 32Si, 32P, 33P, 36Cl, 37Ar, 39Ar, and 81Kr. Interesting to note, vehicle shielding may also generate secondary particles, representing an important component of the radiation present inside the space vehicles. Technically, these secondary cosmic rays are neither "rays" nor "cosmic" (even if indirectly produced by real cosmic rays): they are just particles. In addition, some level of neutron exposure occurs in the spacecraft but currently the contribution of neutrons to the total dose is considered to be small.

17.1.3 The Radiation Level is Linked to the Solar Activity

The sun has an 11-year cycle activity which means that the basic dipole structure of the sun's magnetic field reverses polarity approximately every 11 years. Close to the reversal, at "solar minimum", there are few sunspots and the magnetic field extending throughout the solar system is relatively weak. During a solar maximum, on the contrary, the number of sun spots is high, the solar wind is also stronger, and the sun is slightly brighter (about 0.1%). When the solar magnetic field is stronger, the paths of the electrically charged ions are deflected further and less GCRs reach the earth. Thus, solar maximum cause a radiation minimum and, conversely, during a solar minimum, the magnetic field is weaker and the radiation maximum. Occasionally, a disturbance in the sun's atmosphere, known as a SPE, leads to a surge of radiation particles produced by sudden sporadic releases of energy into the solar atmosphere (solar flares) and by coronal mass ejections, but these are usually of insufficient

energy to contribute to an increase in the radiation field. The strong magnetic disturbance associated with SPE can lead to significant decreases in GCR dose rates. At commercial aircraft operating altitudes, it is so far not currently possible to predict the significant increases in radiation dose rates induced by SPE.

17.1.4 Solar Cycle, Space Mission Duration, and Shielding Determine the Radiation Characteristics

The relative importance of each of these solar components to the radiation burden of crew members of a given space mission also depends on many mission details, for example, the spacecraft trajectory, the time the mission occurs during the solar cycle, the mission duration, and the available shielding within the spacecraft. It is assumed that during a 2.5-year mission to Mars, astronauts may receive a total dose of about 1–2 Sieverts (Sv, being the unit of dose equivalent radiation) while the typical dose received on Earth is around 2.5 mSv/year. For long-duration space missions in deep space, the radiation dose being then directly proportional to exposure time, the challenge will be to find adequate protection to limit the primary as well as secondary cosmic ray exposure.

17.2 Radiation Dosimetry

17.2.1 Determination of the Radiation Dose

Radiation dosimetry on board space crafts is necessary for different reasons. First, astronauts are subjected to an operational radiation safety program, including a dosimetry procedure and system to estimate their cumulative equivalent doses received during each spaceflight (NCRP 2002). Second, different experiments and instruments on board the spacecraft can be affected by the space radiation. Radiation dose rates can vary by a factor of 2 within the space craft, dependent upon the shielding and the orientation of the space craft. In addition, there is also the variation in time due to the solar cycle and SPE, so it is necessary to have a continuous monitoring program for radiation doses.

Determination of radiation doses in space is not straightforward because the space radiation spectrum is very complex and different from the situation on Earth. As mentioned earlier, radiation dosimetry for humans is expressed in dose equivalent radiation (expressed in Sieverts or Sv). As an example one can say that astronauts in low Earth orbit, such as the International Space Station (ISS), receive between 200 and 600 μSv/day, dependent on many factors. For comparison, the natural background from external radiation on the Earth's surface is between 2 and 3 μSv/day. During SPE the dose to astronauts can be very high, even several mSv or higher, depending on the strength of the eruption.

The radiation effects are also dependent on the LET (which is a physical measure of the energy transfer) value of the radiation particle.

17.2.2 Space-related Constraints in Radiation Dosimetry Instrumentation and Calculations

The instruments used for radiation dosimetry in space are bound by some constraints (Benton and Benton 2001). Because of the high cost of launching equipment into orbit, radiation detectors must be small and of low mass. Furthermore, they should be of a robust design, and able to withstand long period of use without failing. Finally, they need to consume as little power as possible. The types of materials that can be used are also bound by constraints on crew safety, such as the possible outgassing of certain polymers and the limited bandwidth available for the transmission of data to Earth. From a technological point of view it is very challenging to measure the large dynamic range of energies, fluxes, and particle types. During SPE the fluxes can increase by several orders of magnitude. It is desirable to have a good charge, energy, and LET resolution in order to measure the appropriate dose quantities. Currently there is no detector that can fulfil all these requirements, therefore the results from different detectors need to be combined.

Besides measurements, there is also an important role for calculations. In this respect, radiation transport calculations which permit the study of the motion and interaction of radiation with materials when combined with measurements results give a better estimate of the dose limit quantities. Environmental and shielding models are available for the evaluation of the radiation environment in situations such as the ISS. However, these codes are not yet accurate enough to rely solely on them, and there is a real risk that high transient radiation doses will occasionally occur, particularly from SPE and relativistic electrons from the outer radiation belt. These can cause problems for astronauts, but also for the electronics on board spacecraft and for experiments running on board. Measurements with area monitors and personal dosimeters should be used for determining the doses and for normalizing radiation transport calculations, as well as for real-time estimates of dose rates for As Low As Reasonably Achievable (ALARA) purposes.

17.2.3 Passive and Active Radiation Detectors

Cosmic rays can be detected directly when they pass through radiation detectors flown aboard satellites or in high altitude balloons. Ionising radiations interact with the detector, the detector converts this interaction into a signal which can be amplified and interpreted by other devices. Detectors can be passive detectors (which require a subsequent process to determine useful information, they thus need to be "read" at a later stage in order to ascertain the level of exposure recorded), or active detectors (which enable real time information to be obtained).

Three types of passive detectors are mainly used for space crew and area monitoring: thermoluminescent detectors (TLDs), solid state track etched detectors

(TEDs), and optically stimulated luminescent detectors (OSLDs) (Yukihara et al. 2006; Berger and Hajek 2008).

- The TLD consists of a crystal (e.g., CaSO4) which gives off light when heated, the light being proportional to the degree of radiation exposure observed by the TLD. The crystal is usually placed in a holder that contains filters which can be used to differentiate between skin doses and penetrating doses of ionising radiation.
- The TED is a section of a solid material (crystal, glass, or plastic) that is directly exposed to nuclear radiation. For analysis, the TED is etched, and inspected microscopically. The pathways of nuclear particles give information regarding the charge, mass, direction of motion of the particles as well as the energy. The benefits over other radiation detectors include the knowledge about the distinctive particles.
- The OSLD makes use of electrons trapped in the forbidden band in the crystalline structure of certain types of matter (such as aluminium oxide).
- The disadvantage of TLDs and OSLDs is that they do normally not provide information on the dose equivalent (or on the LET). However, since different types of TLDs and OSLDs show different behavior for high LET radiation, a combination of different types can give extra information about the high LET region (Hajek et al. 2008). A system based on board TLD measurements was developed for the Mir station. With this system, TLDs could be read out, annealed, and reused on board (Apathy et al. 2007).

The three kinds of passive detectors are often used in combination (Benton and Benton 2001; Vanhavere et al. 2008) to measure the complete range of radiation in space.

Most of the active detectors are ion chamber-based proportional counters and solid state charged particle telescopes. Recent technological advances such as reduction in size and power consumption (microelectronics), increased data storage capabilities, and longer lived batteries have made active detectors more practical. However, high LET particles can only be measured if they are stopped completely inside the detectors. Therefore, the detectors have to be arranged in a telescopic configuration, which limits the solid angle sensitivity. Most useful data are measured with a so-called Tissue Equivalent Proportional Counter (Shinn et al. 1999; Zhou et al. 2007). This detector simulates a 1 μm diameter biological cell and measures the energy deposition in this volume, thereby estimating the LET distribution. Different Si-based spectrometers have been, and are still, active in the ISS, and provide LET spectra: the most important examples are the DOSTEL instrument (Beaujean et al. 2002), the RRMD (Sakaguchi et al. 1999), the CPDS (Badhwar et al. 1995), and the Liulin (Dachev 2009). The response characteristics of all types of devices should be determined prior to use. This is accomplished by a combination of both experiment and calculation. Many of the detectors, both passive and active, participate in an international series of common characterization and calibration exercises called ICCHIBAN (Yasuda et al. 2006).

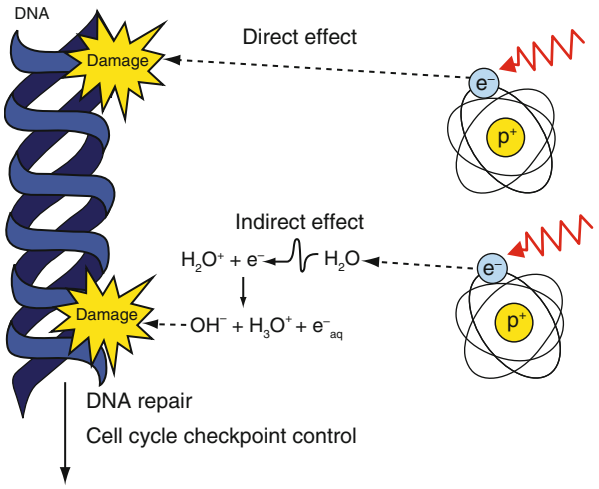

Fig. 17.1 Ionizing radiation induces direct DNA damage and indirect damage through the radiolysis of water (figure is *adapted* from a figure in (Morgan and Sowa 2007))

17.3 Radiobiology

17.3.1 Introduction

All biological consequences of ionizing radiation on living tissues are a result of the interaction with atoms in a process called ionization. Ionizing radiation has sufficient energy to remove one orbital electron from an atom, thereby creating an ion pair. There are two mechanisms by which radiation ultimately affects cells: either by direct ionization of the target molecule, or indirectly by the production of free radicals which may ultimately affect the target molecule (Fig. 17.1). During direct ionization, radiation transfers energy directly to the atoms of critical cellular components such as DNA. The mean number of inactivated or damaged molecules is directly proportional to the radiation dose. If a cell is exposed to ionizing radiation, the probability of directly interacting with DNA is low, as it makes up only a small part (1%) of the cell. The main constituent of all cells of the human body is water (up to 80%). Therefore, most of the energy produced by ionizing radiation (particularly low-LET) leads to water radiolysis, which results in free radical production that ultimately can lead to indirect DNA damage. Free radicals are highly reactive compounds, such as hydroxyl radicals and superoxide anions, which are characterized by an unpaired electron. In addition, other reactive compounds including hypochlorite ions, hydrogen peroxide and hydroxyl ions can also be produced.

Radiation induces a large spectrum of DNA lesions including single strand breaks (SSB), double strand breaks (DSB), base loss, base changes, and cross-links. The DSB constitute the principle cytotoxic lesion in response to both low- and high-LET ionizing radiation, and are considered to be the critical primary lesion in the formation of chromosomal aberrations. The quantification of chromosomal aberrations

after radiation exposure is frequently used as biological dosimeter or to evaluate radiosensitivity of individuals (see Chap. 25). Substantial evidence indicates that high-LET radiation such as protons or heavy ions induces DNA damage that is quantitatively and qualitatively different from that caused by low-LET photons (Fakir et al. 2006). High-LET radiation produces dense ionization tracks, thereby inducing a greater number of, and more complex, "clustered" DNA lesions than low-LET radiation (Hada and Georgakilas 2008; Terato et al. 2008). These clusters contain various types of DNA damage (e.g., SSB, DSB) within a localized region of the DNA molecule and are associated with the increased relative biological effectiveness (RBE) of high-LET radiation beams (Fakir et al. 2006; Hada and Sutherland 2006).

The global response of a cell to DNA damage triggers multiple pathways involved in sensing DNA damage, activating cell cycle checkpoints and inducing DNA repair (Su 2006). However, when the damage is severe, cellular apoptosis can be induced. Failure of DSB repair or misrepair can initiate genomic instability, causing chromosome aberrations and genetic mutations, and may eventually lead to cancer. Besides damaging the DNA molecule, ionizing radiation can cause a number of lesions in other macromolecules as well (e.g., lipid peroxidation, reactive oxygen species). These non-DNA lesions trigger multiple signalling pathways including Protein Kinase C (PKC), MAP Kinase (MAPK), and JNK, which are involved in cell cycle control, DNA repair, and apoptosis. Recently, other radiation-induced phenomena have been described. These include nontargeted and delayed effects such as bystander effects, genomic instability, and adaptive response (Averbeck 2010). Bystander effects occur in cells that are not hit directly, but which are affected by signals derived from neighboring irradiated cells. Genomic instability is characterized by the increased rate of acquisition of genomic alterations (e.g., chromosomal aberrations, mutations) of the progeny of an originally irradiated cell appearing several generations after irradiation. On the other hand, the adaptive response model postulates that certain doses of low-dose radiation may be beneficial, and renders cells less susceptible to the damaging effects of radiation.

17.3.2 Biological Effects of Radiation

17.3.2.1 Deterministic and Stochastic Effects of Ionizing Radiation

Radiation risk assessment by advisory bodies such as ICRP (International Commission on Radiological Protection) and NCRP (National Council on Radiation Protection & Measurements) have classified biological effects of radiation as either deterministic or stochastic. *Deterministic* effects are associated with high doses of radiation exposure over a short period of time. The severity of the effects in affected individuals depends on the dose and increases with the magnitude of the radiation. There is a threshold radiation dose, below which the deterministic effect has, so far, not been detected clinically (Fig. 17.2a). *Stochastic* effects are usually associated with exposure to low doses of radiation over a longer period of time, typically like those encountered by astronauts on board the ISS. The probability of inducing the effect, but not the severity of the effect, is dose-related (Fig. 17.2b). Dose-effect

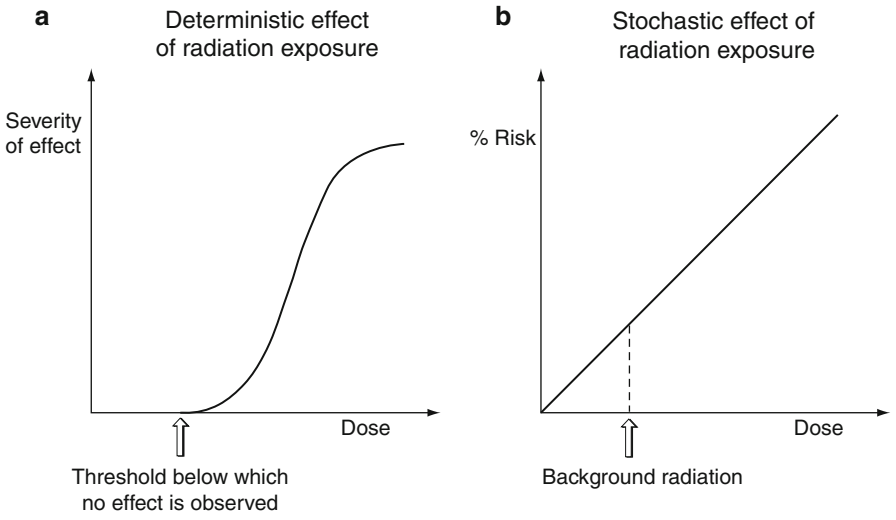

Fig. 17.2 Biological effects of ionizing radiation

curves for these changes are considered to be nonthreshold in type. It is assumed that there is always a small probability of an effect even at very low doses. Low doses can be defined as a dose, and dose rate, at which, on the average, only a fraction of all targets (cell nuclei) is affected by an energy deposition event. In this dose range, the risk for one cell to be transformed is very low. However, the risk to the organism of having one transformed cell depends on the number of cells being hit. The upper limit according to this criterion is 0.020 Gy (ICRP). Increased incidence of cancer after low-dose radiation is an example of a stochastic effect. For the induction of tumors, doses below 0.1 Gy are considered as low doses. The linear no-threshold model presupposes that the damage caused by ionizing radiation linearly increases in response to the dose. Furthermore, nontargeted as well delayed effects including bystander effects, genomic instability, and adaptive response might be involved in the response to low doses.

17.3.2.2 Early and Late Effects

Radiation effects can appear rapidly, but also after a delay. *Early* or *acute effects* are those occurring within hours, days, or a few weeks following high-dose exposure (>1 Gy) in a short period of time. These effects appear to be threshold phenomena and are therefore always classified as *deterministic*. Exposure to high doses of ionizing radiation can cause a rapid whole-body response, often referred to as acute radiation syndrome (ARS) or radiation sickness. These high doses tend to kill cells in such a way that tissues and organs become damaged and their functioning impaired. The principal sites of biological action are rapidly proliferating cells from the bone marrow, gastrointestinal cells, skin, and testes. These tissues and organs are classified as radiosensitive organs. ARS is characterized by a sequence of phased symptoms in which the clinical effects develop proportionally to the dose amount

(Xiao and Whitnall 2009). Moderate doses (1–7 Gy in humans) induce depression of bone marrow function, known as hematopoietic syndrome. This syndrome leads to decreased resistance to infections and hemorrhage. Higher single doses (about 8 Gy or more) will result in gastrointestinal syndrome leading to small intestinal cell killing. Very severe whole-body exposure (20–40 Gy) is characterized by a deteriorating state of consciousness with eventual coma and death (neurovascular syndrome).

Late or *long-term effects* usually occur after a number of months or years following radiation exposure. For radiation protection purposes, these late effects are classified as being stochastic or deterministic. Epidemiological studies have clearly identified an increased risk of several types of cancers induced by ionizing radiation (Preston et al. 2007), rendering *carcinogenesis* as the main somatic *stochastic late effect*. It is generally agreed that DNA damage, including DSB, mutations, and chromosomal rearrangements are the initiating event in the multistep process leading to malignant transformation. In this context, radiation-induced chromosomal aberrations in peripheral blood lymphocytes are considered as a validated biomarker of cancer risk estimation (Durante 2005; Norppa et al. 2006; Boffetta et al. 2007). In addition to an increased risk of carcinogenesis, long-term immune dysfunction should also not be underestimated (Kusunoki and Hayashi 2008). A classic example of a *deterministic late effect* induced by ionizing radiation exposure is cataract formation (reviewed in Ainsbury et al. 2009). The eye, and more specifically the crystalline lens, is one of the most radiosensitive organs of the human body, and therefore a most sensitive target for deterministic effects. Ocular ionizing radiation exposure causes dose-related, progressive changes in the lens, finally leading to cataract. The ICRP and NCRP have reported threshold values for visually disabling cataracts of 2–10 Gy for single brief exposures and >8 Gy for chronic exposures. Furthermore, apart from lens opacity, it has long been realized that high-radiation doses also have the potential to cause effects such as cardiovascular diseases as well as cognitive impairment and that such noncancer effects at low doses cannot be readily explained by the extrapolation of cancer risk from high to low doses (LNT model), pointing out the need for more experimental (mechanistic) and epidemiological studies to address this particular extrapolation issue to radiation-induced noncancer effects.

17.4 Biological Effects of Cosmic Radiation

Compared to individuals on Earth, astronauts receive much higher doses of ionizing radiation during spaceflight. Dose estimates for interplanetary space travel indicate that astronauts receive 0.5–1.4 Gy/year. Estimates for a round-trip to Mars show that total cumulative doses of up to 2–3 Sv are likely (Cucinotta et al. 2001). However, these doses are not expected to result in significant acute radiation effects (thereby assuming no significant SPEs during the mission), but they may increase the long-term health risks that are associated with radiation exposure. Besides this, the type of radiation in space differs from the typical low-LET terrestrial radiation (e.g., X-rays, γ-rays), and consists mainly of protons and HZE particles (e.g., iron,

oxygen, carbon, and silicon ions). Although protons account for >90% of deep space radiation, charged particles are predicted to account for most of the biological consequences of cosmic radiation exposure, due to their high-LET and their linear track structure (Swenberg et al. 1991; Green et al. 2001). Unfortunately, shielding against this type of ionizing radiation is not feasible. Exposure to high-LET radiation is therefore considered as a major health risk for astronauts and is of major concern during long-term spaceflights.

Cataract formation was the first proven late deterministic effect of space radiation on astronauts. An increased risk of cataract with lens doses greater than 8 mGy was observed in astronauts (Cucinotta et al. 2001; Rastegar et al. 2002; Chylack et al. 2009). The induction of late stochastic effects such as cancer risk after cosmic radiation exposure is so far unknown (Cucinotta and Durante 2006; Durante and Cucinotta 2008). Estimations of cancer risk for space missions are mainly based on epidemiological settings from data obtained from atomic bomb survivors and patients exposed to radiation therapy (Little 2009). However, most of these studies are related to low-LET radiations (e.g., X-rays, γ-rays), and do not take into account the important biological differences that exist between low-LET and high-LET radiations. As a result, extrapolation of low-LET data for radiation risk assessment on astronauts can lead to wrong conclusions (Cucinotta and Durante 2006). Recently, ground-based studies on animals showed that exposure to high-LET radiation has a higher carcinogenic effect compared to low-LET (Trani et al. 2010). Further studies in this field will definitely contribute in reducing the present uncertainties associated with cancer risk estimates on astronauts. Besides experimental research, the increased use of charged particles (protons and carbon ions) for radiotherapy purposes (hadrontherapy) can increase our general knowledge about the biological effects of heavy ions in general.

As mentioned before, measurement of chromosomal aberration frequencies in lymphocytes after radiation exposure have been proposed as an indicator of cancer risk (Durante 2005). So far, several papers have described radiation-induced chromosomal damage in astronauts' lymphocytes (Testard et al. 1996; Obe et al. 1997; Yang et al. 1997; George et al. 2001; Durante et al. 2003; Greco et al. 2003; George et al. 2005). Short-duration missions did not result in significant detectable differences in chromosome abnormalities measured before and after flight. In contrast, an increase in postflight aberrations was found in the case of long-term flights. In a study by Durante (2003), however, no correlation was found between aberration frequencies and total mission duration or in the cumulative dose equivalent in space for 13 crew members involved in multiple spaceflights. In addition, it appeared that the postflight decline in time of chromosomal aberrations was faster than expected and significant heterogeneity was observed among individuals. Besides differences in individual radiosensitivity, the adaptive response mechanism to space radiation might explain these observations. This adaptation might take place after the first exposure, leading to an increased radioresistance in exposed individuals. This effect can explain the lack of adverse health effects in (very) high background radiation regions on Earth. The long-term consequences of these persistent chromosomal rearrangements are not well understood so far, and further investigation in this field is definitely needed.

17.4.1 Immune Dysfunction in Space: Impact of Cosmic Radiation?

Immune dysfunction during spaceflight is of paramount concern and can lead to serious health problems. The first study about immune abnormalities in space dates back to the 1970s, where reduced reactivity of peripheral blood lymphocytes in crew members was observed (Konstantinova et al. 1973). Currently, numerous immune aberrations have been reported in astronauts, cosmonauts, and animals flown in space (reviewed in Gueguinou et al. 2009). Some of these observed alterations include variations in peripheral blood leukocyte populations, changes in function of cells involved in innate immunity, decreased cytokine expression, depression of T cell activation and proliferation, and many others. So far, the precise nature of immune dysregulation related to spaceflight is unknown (see Chaps. 9–14) and multiple underlying causes might be involved. In this context, the impact of cosmic radiation, which is always present during spaceflight, should not be underestimated.

To gain more insight into the specific influence of radiation on the immune system, Earth-based experiments are used increasingly and represent a more reproducible alternative to in-flight experiments. To date, several ground-based studies in animals demonstrated that ionizing radiation influences many aspects of the immune system and can cause immune dysfunction. Until recently, the majority of radiobiology studies were related to low-LET radiation such as X- and γ-rays (Harrington et al. 1997; Shankar et al. 1999; Gridley et al. 2001; Pecaut et al. 2001; Shearer et al. 2005). However, due to the recent worldwide increase in particle accelerator facilities, several studies investigated the effect of whole-body exposure to high-LET radiation including protons and heavy ions. In this way, it is possible to expose cells and animals to types of radiation encountered by astronauts during interplanetary space travel. Laboratory studies have shown that whole-body radiation of rodents can result in significant acute and long-term effects on the immune system. Table 17.1 gives a summary of some important papers describing ground-based experiments that investigate immune changes after acute and chronic exposure to low-and high-LET radiation.

17.4.1.1 Acute Effects

In general, when mice are exposed to a single exposure of up to 3 Gy of protons, photons, or high-LET particles, a clear effect on different immune cell populations is observed. There is a large dose response on day 4 (acute) followed by general recovery over the following 2–3 weeks. Independent of the radiation type, relative radiosensitivities of the various lymphocyte populations (B cells > T cells > NK cells) and T cell subsets (CD8+ > CD4+) are observed (Kajioka et al. 1999, 2000; Gridley and Pecaut 2006; Pecaut et al. 2006; Gridley et al. 2008b; Pecaut and Gridley 2008). To better mimic the space radiation environment, experiments with simulated solar particle event (sSPE) were performed (Gridley et al. 2008b). For this purpose, mice were exposed to a high dose of sSPE protons over a period of 36 h. The results obtained were compared to γ-ray and proton exposure with the same dose. Acute effects including general immune depression and leukocyte abnormalities were present; however, the damaging effects of sSPE on leukocytes

Table 17.1 Ground-based studies – impact of low- and high-LET radiation on the immune system of rodents

Reference	Irradiation source	Animal model	Time point	Effects
Harington et al. (1997)	γ-rays: 1–7 Gy	C57Bl/6 mice	Acute (1–4–7 days)	Immune suppression
Kajioka et al. (1999, 2000)	Proton: 3 Gy (0.4 Gy/min) γ-rays: 3 Gy	C57Bl/6 mice	Acute (4–10–15–29 days)	Decrease in lymphocyte populations → B sens>CD4>CD8>NK resist Decrease in acute response to antigen No significant differences between proton and γ-ray exposure
Pecaut et al. (2001)	γ-rays: 0–0.5–1.5–3 Gy LDR: 1c Gy/min HDR: 80c Gy/min	C57Bl/6 mice	Acute (4 days)	Decrease in lymphocytes populations B sens>CD4>CD8>NK resist Immune changes depend more on dose than on dose rate
Gridley et al. (2001)	γ-rays: 0–0.5–1.5–3 Gy LDR: 1c Gy/min HDR: 80c Gy/min	C57Bl/6 mice	Acute (4 days)	Decrease in number of blood cells Decrease in IL-2 secretion by activated spleen cells Changes depend more on the dose, than on the dose rate
Gridley et al. (2002a)	Proton: 0–0.5–1.5–3 Gy (entry region Bragg peak) LDR: 1c Gy/min HDR: 80c Gy/min γ-rays: 3 Gy	C57Bl/6 mice	Acute (4 days)	Decrease in number of lymphocytes NK are more radioresistant Significant dose and dose rate effects Effect of proton irradiation (3 Gy) is larger than γ-ray exposure (3 Gy)

Pecaut et al. (2002)	Proton: 0–0.5–1.5–3 Gy (entry region Bragg peak) LDR: 1c Gy/min HDR: 80c Gy/min γ-rays: 3 Gy	C57Bl/6 mice	Acute (4 days)	Decreased splenocyte response Increased blastogenesis Effects depend on dose (and not dose rate) Effects are more pronounced with proton exposure compared to γ-rays
Gridley et al. (2002b)	Fe ion: 0.1–0.5–2.0 Gy (LET = 148.2 keV/μm) Si ion: 2.0 Gy (LET = 42.1 keV/μm)	C57Bl/6 mice	Acute (Fe ion: 4 days) Chronic (Fe ion, Si ion: 113 days)	Acute effects: decrease in lymphocytes(B sens > CD8 > CD4 > NK resist) Chronic effects after Fe exposure: high number of B cells, low number of CD8 cells Chronic effects after Si exposure: low number of NK cells Immune aberrations persist long after exposure Effects depend on radiation type
Pecaut et al. (2003)	Proton: 3–4 Gy (+/−shielding)	C57Bl/6 mice	Chronic (122 days)	Dose-dependent decrease in lymphocyte populations
Shearer et al. (2005)	γ-rays: 0.3 Gy	Balb/c mice		Decrease in number of immune cells
Pecaut et al. (2006)	Fe ion: 0–0.5–2–3 Gy (LET = 148.2 keV)	C57Bl/6 mice	Acute (4 days)	Decrease in number of lymphocyte populations B sens > CD8 > CD4 > NK resist
Gridley et al. (2006)	Fe ion: 0–0.5–2–3 Gy (LET = 148.2 keV)	C57Bl/6 mice	Acute (4 days)	Alterations in leukocyte response and function

(continued)

Table 17.1 (continued)

Reference	Irradiation source	Animal model	Time point	Effects
Gridley et al. (2006)	γ-rays: 2 Gy Proton: 2 Gy C ion: 2 Gy (LET = 12.9 keV/μm) Fe ion: 2 Gy (151.5 keV/μm)	C57Bl/6 mice	Chronic (110 days)	Significant aberrations in immune parameters observed 4 months after exposure
Gridley et al. (2008a)	Fe ion: 0–1–2–4 Gy (LET = 148.2 keV/μm)	SD Rat	Chronic (9 months)	Decrease in number of lymphocytes
Gridley et al. (2008b)	SPE protons: 2 Gy (chronic) Protons: 2 Gy (acute) γ-rays: 2 Gy (acute)	C57Bl/6 mice	Acute (4–21 days)	Effects on immune system with SPE protons less pronounced compared to other types of radiation (acute γ-rays or acute protons)
Gridley et al. (2009)	Proton: 0.01–0.05–0.1 Gy LDR: 0.1 c Gy/h (delivered over a 2 week period)	C57Bl/6 mice	Acute (4–21 days)	Changes in CD4 T cell gene expression after low-dose proton irradiation. LDR enhances CD4 T cell responsiveness
Pecaut and Gridley (2010)	Fe ion: 0.5–2–3 Gy	C57Bl/6 mice CBA/Ca	Acute (4–30 days)	Mouse strain influences Fe radio-immune response
Gridley et al. (2010)	+/−SPE protons: 1.7 Gy (chronic) +/−pre exposure to γ-rays: 0.01 Gy (LDR: 0.179 mGy/h)	C57Bl/6 mice	Acute (4–21 days)	Pre-exposure to LDR photons does not protect against adverse effects of radiation mimicking a SPE on the immune system
Rizvi et al. (2011)	+/−SPE protons: 1.7 Gy (chronic) +/−pre exposure to γ-rays: 0.01 Gy (LDR: 0.179 mGy/h)	C57Bl/6 mice	Acute (4–21 days)	Protracted exposure to LDR γ-rays modifies effect of SPE protons on lymphocyte signalling proteins and secretion of cytokines

were generally less pronounced compared to the acute photon and proton radiation. Besides the risk of relatively high-dose exposure (e.g., during an SPE), low dose/low-dose-rate (LDR) radiation must be taken into account when performing research in the context of radiation risks for astronauts. Very recently, Rizvi et al. (2011) demonstrated that LDR γ-radiation can modify the response of the $CD4^+$ cell exposed to sSPE protons, thereby increasing cellular tolerance.

In the context of individual radiosensitivity, a recent paper compared the potential impact of mouse strains with a genetic background on various immune parameters after acute iron ion exposure (Pecaut and Gridley 2010) and showed that the impact of the genetic background on radiation-induced immune aberrations appeared to be minimal, and only included changes in circulating phagocytic populations, erythrocytes, and liver mass.

17.4.1.2 Chronic Effects

So far, knowledge about the long-term effects on the immune system after exposure to different types of radiation is limited (Gridley et al. 2002b, 2008a; Pecaut et al. 2003; Gridley and Pecaut 2006). Summarized in the following table, these studies investigated the potential chronic effects on lymphoid cells 4 months after exposure to a single high dose of photons, protons, iron, silicon, or carbon ion whole-body irradiation (Gridley et al. 2002a; Pecaut et al. 2003; Gridley and Pecaut 2006). The first study in this field investigated long-term effects (3 months) after iron and silicon irradiation (Gridley et al. 2002b). In response to Fe ion irradiation animals had significantly increased total lymphocyte and B cell numbers, whereas $CD8^+$ T cell proportions were low, compared with nonirradiated controls. However, whether these changes result in abnormal/compromised immune responses is not clear. Interestingly, these changes could not be observed after exposure to Si ion beams. Long-term changes in mice exposed to Si beams resulted in a lower number of NK cells. After whole-body exposure to proton irradiation at doses of the order of large SPE, dose-dependent decreases in $CD8^+$ and NK cells were observed (= depression of peripheral white blood cell count) (Pecaut et al. 2003). Another study showed increases in the number of T cells and a decrease in NK cells in response to proton and carbon ions (Gridley and Pecaut 2006). Finally, a recent study by Gridley (2008b) focused on the impact of a single iron ion 9 months after whole-body irradiation in rats and showed lower numbers of circulating lymphocytes and monocytes, indicating that the intrinsic quality of a particle beam is of importance as well, and can evoke different long-term effects on the immune system.

In summary, several ground-based studies clearly demonstrated acute and long-term changes in the immune system status of whole-body irradiated animals exposed to low-LET and/or high-LET radiation. Some of these observed alterations include changes in the numbers of T- and NK cells which are important cellular components to suppress infections and kill virus-infected or neoplastic cells. Prolonged deficiency in any of these lymphocyte populations can have serious consequences for astronaut health. However, it still needs to be determined whether these observed immunological aberrations will actually result in impaired immune function.

Another important observation in this field is that results obtained with high-LET are not always similar to those obtained after low-LET radiation such as X-rays and γ-rays. This clearly demonstrates that caution is important when extrapolating to low-LET results. In addition, long-term immune changes differ significantly between the various high-LET particles, thereby indicating that the intrinsic quality of the particle beam may be important as well.

Ground-based experiments are increasingly being used and represent a more reproducible alternative to in-flight experiments. Although these experiments are a good model to investigate the impact of radiation on the immune system, they most often evaluate the effect of exposure from a single source of radiation. In this context, simultaneous exposure to radiation of different types, thereby better simulating the radiation spectrum to which astronauts are exposed to, might be interesting to decipher whether this might affect a broader range of immunological parameters.

17.4.1.3 Spaceflight-associated Immune Changes: Examples of Tentative Interaction Between Radiation and Other Space Flight Stressors

To date, it is beyond doubt that spaceflight can induce changes in the immune system. Most, if not all, of these immune alterations have been attributed to both psychological stress and the microgravity (μG) environment. In the context of cosmic ray exposure, it is only after long-term missions that increased levels of cosmic radiation may play a more significant role in this immune dysfunction. However, it is likely that several of the space stressors can interact with one another. These interactions may be additive or synergistic, but can be antagonistic as well, thereby resulting in a final common effect on the immune system that might compromise astronaut resistance to infections and other diseases (see Chaps. 15 and 16). In the next paragraph an example of a tentative interaction between two spaceflight specific stressors is given.

17.4.1.4 The Combined Effects of μG and Radiation

During spaceflight, μG induces numerous systemic effects including alterations in the musculoskeletal system, cardiovascular system, sensory-motor system, and immune system. With regard to the latter, changes such as decreased number and responsiveness of T lymphocytes, reduced cytotoxic activity of NK cells, and alterations in cytokine and chemokine activity have been reported (reviewed by Blaber et al. 2010). However, one might ask whether this reduced gravity can alter the cellular response to ionizing radiation. Experiments performed on living embryonic systems in space showed a synergistic interaction between both space stressors thereby decreasing cell survival and inducing chromosomal aberrations (Reitz et al. 1989; Horneck 1999). However, further studies demonstrated that the interplay between both could not be explained by a decreased capacity to repair damaged DNA (Kiefer and Pross 1999; Pross et al. 2000). Therefore, other mechanisms have to be postulated for this synergism. One may hypothesize that immune cells respond to decreased gravity as well as to radiation challenges by activating similar cell signalling pathways (see Chap. 14). In the context of μG, “gravi-sensitive” signal

transduction components are present in different cell compartments of immune cells such as on the cell surface (e.g., IL-2 receptor, which can result in a diminished proliferative response of T cells), in the cell cytoplasm (e.g., intracellular signalling pathways), and in the nucleus (e.g., expression of the genes regulating a number of cellular processes including differentiation and proliferation) (Ullrich et al. 2008). With regard to intracellular signalling pathways, various kinases such as tyrosine, PKC, and MAPK play important roles in response to μG. Besides μG, it has been shown that radiation also induces changes in the activation of different kinases in immune cells (Varadkar et al. 2003; Varadkar and Krishna 2004; Mitra et al. 2007). Tyrosine kinase, PKC, and MAPK activity increase with increasing dose after irradiation in lymphocytes in vivo. However, in contrast, MAPK activity decreased with an increasing dose in ex vivo irradiated lymphocytes. The effect might become even more complicated when comparing low-LET data with results obtained after high-LET radiation (Narang et al. 2009). The importance of the radiation-counterpart in cellular changes in response to physical space stressors such as μG is still not clear, and more research is definitely needed to gain additional insight into this complex matter. Moreover, the differences between the response of a single cell and the one of the whole animal must be considered as well. Once again, several studies underscore that in vitro data cannot be extrapolated indiscriminately to in vivo conditions.

Besides similar cell signalling pathways, μG and radiation can indirectly affect inflammation by influencing components that are essential in mediating the inflammatory response. A crucial step in inflammation is the trafficking of leukocytes from the blood stream into the tissue. This leukocyte–endothelial adhesion involves dynamic interactions between leukocytes and endothelial cells, and is mediated by several families of cell adhesion molecules (CAMs). CAMs that are expressed on the surface of vascular endothelial cells include the selectin family (E-selectin and P-selectin) and the Ig superfamily (e.g., ICAM-1). These CAMs interact with leukocytes to initiate cell extravasation and migration. The impact of both radiation and μG on the induction of cell adhesion molecules on endothelial cells has been studied (Hallahan et al. 1996; Romanov et al. 2001; Zhang et al. 2008). Both E-selectin and ICAM-1 are increased after X-irradiation, whereas VCAM and ICAM-1 increase under hypogravity conditions. These experiments indicate that leukocyte adhesion (and consequently inflammation) might be promoted both by radiation and μG. Additional experiments are needed to gain more insight into the combined effects of both physical spaceflight stressors.

To gain more insight into the potential interplay between these two physical space stressors in immune cells, Earth-based experiments that simulate space conditions can be useful. In this light, μG-simulating devices such as rotating-wall bioreactors (clinostat) or the Random Positioning Machine (RPM) are currently used to perform in vitro experiments under hypogravity conditions. Ideally, cells should be exposed to μG and radiation at the same time. However, to our knowledge, virtually no such studies have yet been performed in which cells are simultaneously exposed to both these physical space stressors. At SCK•CEN (Belgium), experiments are currently performed in which human lymphocytes are exposed simultaneously to both μG and radiation.

17.5 Conclusion

It has become clear that several aspects of the spaceflight environment lead to acute and long-term changes in the immune system. Therefore, to reduce the health risks for astronauts, it is important to better understand the mechanisms responsible for the changes observed in the immune parameters. Ideally, to discriminate between different factors and their impact on the immune system, data should be obtained e.g., from the same cohort of animals that are exposed simultaneously to different spaceflight stressors. However, various experimental set-ups (space vs. Earth-based models) are concomitant with different uncontrolled stressors e.g., shipping animals, housing. These conditions may elicit immune system alterations that always make direct comparison problematic. In addition, to study the effect of space radiation on ground-based models, radiation should be delivered at a very low dose rate for extended periods in order to be as relevant as possible to spaceflight. Unfortunately, these conditions are not easy to achieve due to facility limitations and high demand for beam time. Currently, researchers are starting to elucidate the different effects of cosmic radiation on the immune system. Nevertheless, several important questions remain: Which pathways are responsible for repairing radiation-induced changes? How capable is the irradiated immune system of responding to an immune challenge? How are other metabolic cofactors (see Chap. 13) and hormones and transmitters affecting this process?

In conclusion, gaining more insight into changes in immune responses after radiation exposure is needed to more accurately predict health risks associated with long-duration spaceflight. This knowledge might not only be relevant to extended ISS missions (1 year+) and to future exploration and colonization missions in space, but also of significant importance to life on Earth and to patient care, e.g., during cancer radiation therapy, when metabolic effects, the immune system, and the radiation effects are interacting closely on the therapeutic goals of curing and limiting cancer growth, respectively.

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Part IV

Preventive and Diagnostic Tools and Strategies

Considerations for Development and Application of Health Monitoring Tools in Space

18

Ines Kaufmann and Alexander Choukèr

18.1 Introduction

Maintenance of health status of astronauts is one of the most important strategic objectives during manned space mission which is necessary to attain the versatile goals of the mission. For astronauts, the next physician is far away as well as problems in radiocircuits with information delay and loss will likely occur. Therefore it is of great interest to develop innovative health care screening tools for astronauts affording to appreciate the certainty of the presence or absence of diseases and to judge the demand of therapeutic strategies. In space, diseases like acute or chronic infections of bacterial, fungal, and viral nature as well as cancer development are of special interest and are strongly linked to the performance of the immune system. Moreover, the latter is eminently modified and compromised as a consequence of stressors in space. Accordingly, monitoring of health in space is also a monitoring of stress and changes of organ functions like immunity.

The pace of development of new diagnostic techniques and (micro-)technologies has greatly accelerated over the past decades (Wilding 2010). Since quality of the diagnostic procedure largely determines quality of care, overcoming deficiencies in standards and methodology deserves high priority. It is self-understanding that it is important to test and to further evaluate the benefits of diagnostic tools on Earth – under laboratory and field conditions – before considering its use in space. This is critical not only because of the limited number of astronauts but also of the high costs of developing the specifically certified hardware for space crafts. The most important challenges result from the fact that after detecting changes in a promising parameter/variable, the description and definition of the latter as a biomarker

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Table 18.1 Examples for noninvasive, minimally invasive, and invasive procedures

Noninvasive procedures
– Cognitive tests
– Monitoring group interaction
– Electrocardiography (ECG)
– Electroencephalography (EEG)
– Temperature (double sensor)
– Breath gas analysis
– Saliva and urine analyses
– Hair analyses
Minimally invasive procedures
– Lab on a chip technologies
– Capillary blood analyses (e.g., analyses by flow cytometry)
Invasive procedures
– Invasive catheterization
– Surgical procedures

(Baumgartner et al. 2010) requires an adequate sample size for either the "sick" or their controls in order to provide the desired information and certainty. Hence it is absolutely necessary to evaluate a diagnostic procedure accurately and extensively with respect to the Good Clinical Practice Guidelines (GCP Guidelines) on Earth before taking into account the use in astronauts during space missions.

18.2 The Ideal Diagnostic Tools for Space

18.2.1 Definitions

Diagnostic investigations collect information to clarify the individual health status. The "ideal" diagnostic tool is able to elevate certainty of the presence or absence of disease – meaning to have high sensitivity and specificity – to support treatment of pathophysiological conditions, to assess prognosis, and to monitor the clinical course (Kottnerus et al. 2002).

Diagnostic procedures to monitor stress effects and health-relevant immune changes must be evaluated not only in accordance with their intended objectives, but also taking into account possible discomfort and complications. The guiding principle for application should be: "Minimize astronauts' risk. Maximize astronauts' care". With regard to the risks and invasiveness of medical procedures, there are three main categories (Table 18.1):

(a) Noninvasive procedures,
(b) Minimally invasive procedures, and
(c) Invasive techniques.

18.2.2 Examples

Medical techniques which do not penetrate the mucosa, skin, or body cavity beyond a natural or artificial body orifice are called *noninvasive*. For centuries, physicians have used many ordinary noninvasive methods rested upon physical parameters. For example, they auscultated heart and lung sounds, percutated or palpated in order to assess organ functions. The discovery of the first modern noninvasive procedures dates back to the end of the nineteenth century: In 1895, the German Physicist Conrad Röntgen discovered a new form of radiation; he called this radiation "X-rays" to denote its unknown structure. X-radiation had the unique property to penetrate through many materials that absorb visible light and to knock electrons loose from atoms. For first time, it was possible to depict anatomical structures and to make a diagnosis in ill patients without exploratory surgery. This was the beginning of a new era. Since then, noninvasive methods have continuously enlarged and have been tested to provide important information on the health of patients in remote areas using real-time telemedicine devices, e.g. an electrocardiogram and ultrasound (Nicogossian et al. 2001; Otto et al. 2009, 2010). Noninvasive methods permit accomplishment of diagnostic procedures without extravagant expenses and with a minimal risk for the individual. They are of outstanding impact for observation of strain and its consequences in individuals under extreme living conditions, by considering stress-associated neurocognitive changes (see for details Chap. 5), by using strategies for psychological (Chap. 19), vegetative (Chaps. 6 and 20) and circadian rhythm changes (Chaps. 7 and 23), respectively. Easily collectable specimens allow the analyses of particular hormones, pharmaceuticals, or contaminants in body fluids (saliva, urine). Fibrous keratin structures (e.g., hair) can also serve as appropriate material to monitor the stress effects (Ballantyne 2007). Moreover, the analyses of volatile organic and anorganic compounds in exhaled air may become an emerging tool (Chap. 21) for maintenance of "crew health", their "system performance" as well as "environmental integrity in space" (Nicogossian et al. 2001), which involves the regular assessment of the microbial environment (Chap. 22) as well. In comparison to minimally or more invasive procedures noninvasive techniques will be the "winners" if they are based on clear data in terms of accuracy, safety, and reproducibility. *Minimally invasive* procedures are characterized by minimizing potentially detrimental diagnostic and/or surgical trauma to the individual. This includes, as an example, peripheral venous or capillary blood withdrawal from the finger tip. Weighing the risks against the benefits of blood draws the benefits will significantly rise, when the number, reliability, and the impact of the readout parameters can support the definition of the individual health status and may direct to appropriate measures for counterbalancing of unfavorable changes (Chaps. 26–30). Analytical tools can involve flow cytometric measurements (Chap. 24) as well as promising point-of-care (POC) diagnostic devices using the lab-on-a-chip technologies (Wilding 2010). An *invasive* procedure penetrates or breaks through the

skin barrier or enters a body cavity, e.g. the mouth, and includes techniques that comprise relevant perforation, an incision, a transection, a catheterization, or another entry into the human body. Invasive techniques are mostly well validated. Their disadvantages are the time-consuming and cost-intensive application requiring the presence of an on-site expert which however can be assisted by telemedicine and robotics in the future as well (Haidegger et al. 2011).

18.2.3 Ready for Space?

In space medicine, the regnant trend is to attach importance to the molecular biology and genetic testing. Both diagnostic tools have significantly amplified the understanding of basics and mechanisms of space-induced health changes. They may possibly add value to select and to appropriately prepare candidates for space flight and to pursue their health status (Chaps. 2 and 25). However, in order to translate these new findings into any improvement of living conditions or relevant impact on health preservation during space missions, it is necessary (1) to clearly determine space-relevant problems in humans, and (2) to differentially monitor and to hereby link the changes observed in one organ to other organ systems. However, especially in space, the implementation of health-monitoring tools will depend on the following factors:

1. Technical feasibility, up-load weight restrictions, and onboard energy consumption,
2. Relevance in respect of health problems occurring in astronauts during missions,
3. Its value for identifying a disease and then monitoring its response to treatment,
4. Manual feasibility by astronauts as well as
5. Safe use and convenience application.

The diversity of diagnostic methods requires a close collaboration of experts in the several fields of medicine and engineering. For the single expert this prevailing trend is a challenge. He has to acquire as much as possible theoretical and practical expertise in the various diagnostic techniques. Hence the goal of education of physicians in space organizations should be to train medical experts who have the competence, the occupational skills, and the experience to take the responsibility for engineering, supervising and referring many diagnostic tests in space. In future, the results of noninvasive or minimally diagnostic procedures could be generated on-site in space and the space crew must be able to autonomously interpret the data and to draw the correct conclusions. This will be only possible if astronauts are trained extensively by medical experts. Alternatively, telemedicine will be established and wireless tools will be provided for astronaut care (“e-health”).

18.3 Summary

“Long-duration missions necessitate further technological breakthroughs in teleoperations and autonomous technology”. This statement made by Nicogoassian and co-workers in 2001 confirms the importance of onboard health care and extends to the critical need, respectively, to make noninvasive or minimally invasive stress and

health-monitoring tools available. These tools should deliver comprehensive data on the degree of stress and health-relevant immune changes of astronauts, their related risks as well to be able to prevent from health threats. To realize the latter in space, existing technical approaches has to be improved and new innovative methods will be developed; this is not only an advantage for the space crew but also equally important for the benefit for patient care on Earth as well.

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Psychological Monitoring

19

Bernd Johannes and Berna van Baarsen

19.1 Monitoring and Preparation Prior to the Mission

When planning a manned mission to space, preparation and training procedures will become more effective when, in addition to technical information and job-related factors, relevant psychological, existential and social aspects are taken into account (Kanas and Manzey 2003; Van Baarsen 2011). The training of astronauts and the preparation of a space mission take several years which is a sufficient time frame to analyze and verify the behavior and performance of individuals and crews.

On the individual level, crew member support should be provided early on in the training period (Baranov et al. 2001). Coping with unknown and perhaps dangerous situations is a unique personal event, for which one must be adequately prepared. On the team level, the preparation period is highly important for the crew development, the intercultural adaptation, and the establishment of mission goals and motivation. Teams perform better when goals originate from and are relevant to them and are evaluated and restructured periodically. Encouragement of reasoning and deliberation among team members may increase group cohesion and decrease the risk of spreading blame and finding scapegoats. Prior companionship and team-talk sessions are of great importance for the development of common goals (Sandal 2001; Tomi 2001). The management of emotions and the building of supportive and constructive relations between the crew (Kass and Kass 2001) have to have been established. Possible effects of social-related personality characteristics can either

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lessen or enhance feelings of loneliness, isolation, boredom and the ability to enjoy one's own company (Van Baarsen et al. 2009). Moreover, the desire to be on one's own, or social anxiety, may influence selection, monitoring, and support programs. These effects certainly stress the necessity to define and to compare different countermeasures and to monitor which ones are most efficient in preventing boredom, distraction, and loneliness (Van Baarsen 2011).

The preparation time provides a good opportunity to support the acceptance of monitoring methods and the acceptance of psychological support tools. The final mission selection is a crew selection with possible individual out-selections and crew rebuilding. One specific crew problem is the late mission assignment of load specialists, for example, from foreign space agencies.

19.2 Monitoring and Support During the Mission

Psychological monitoring and support is most difficult during the mission. However, there are more publications about that mission period than about others (Grigoriev et al. 1985; Kanas 1991; Manzey et al. 1995; Sandal et al. 1996). There are well-documented physical influences of long-term weightlessness on the human body, but health and fitness are not identical with well-being. Well-being depends on how the individual copes with stress due to bodily changes caused by weightlessness, fluctuating O_2 and CO_2 concentrations, radiation exposure, muscle and bone loss, and cardiovascular deconditioning, as well as the adaptation to the psychological effects of long-term isolation. These effects can include asthenia, exhaustion, motivation loss, passivity, feelings of isolation, symptoms of depression, excitability, stimulus deprivation, and movement or activity deficits.

Health maintenance depends much on physical training discipline and hygienic discipline. The sleep–wake cycle within a 24 h day and night cycle as well as the work–rest schedule are disturbed. It is very informative to observe how these daily duties are permanently solved. If there are decreases or changes in spare-time use, such as skipping usual hobbies, this could be an early indication of mood change or motivation loss.

When small teams have to work in highly autonomous conditions, where team members are fully dependent on each other in managing technology and carrying out tasks, and there are communication delays as well as daily (inter)personal and occupational hazards, successful adaptation and adherence to the primary jobs are difficult. Especially when questions of personal goal setting and existential events arise, it is crucial on the one hand to analyze matters of challenge, dedication, and inspiration as they can mean fulfillment. On the other hand, there are loneliness (herein defined as perceived, internalized isolation; Cacioppo et al. 2010), demotivation, and boredom, which can manifest itself as frustration (Van Baarsen 2001, 2011). While positive feelings of personal achievement can increase team performance, coherence and well-being, negative impressions of attainment, failure, and boredom may enhance difficulties with interpersonal and intercultural contacts and communication, supportive behavior and companionship. The availability of intimate contact as well as emotional and instrumental support is an influential social resource which

may attenuate feelings of distress and loneliness (De Jong 1998; Van Baarsen 2002). During powerful isolating events such as a long duration spaceflight, loneliness can be experienced due to changes in the size of the personal network and its interpersonal dynamics as well as the changes in perceived intimacy and support. Particularly members of small intercultural teams are prone to experience loneliness: Increased dependency, different cultural backgrounds, and communication styles (Gushin et al. 2001; Kanas et al. 2000; Palinkas et al. 2004) as well as the limited number of contacts without the possibility of altering the crew composition can result in decreased satisfaction and/or increased need for intimacy and support. In fact, smaller networks discourage social seeking behavior and decrease the chance that emotions can be shared (Van Baarsen and Broese van Groenou 2001). Recent research (Van Baarsen et al. 2009) of the six crew members participating in a pilot study of the MARS500 simulation program (see also Chap. 31) showed an increase in loneliness, particularly shortly after confinement for 105 days, with considerable variation in how crew members adjusted to the confinement in terms of loneliness over time. Loneliness frequently results in a decrease of well-being measured in the form of, for instance, depression, sleeping problems, disturbed appetite, and adaptation disorders (De Jong 1998). Loneliness and poor health, interrelated through a complex process, may result in feelings of alienation and estrangement (Van Baarsen 2002).

Presently, psychological monitoring focuses usually only on performance in special and common tasks, for example, docking training (Hockey 1986; Manzey et al. 1998; Salnitski et al. 2001; Sandal et al. 1996), or have a scientific background. The long-term effect of limited resources in insignificant daily tasks, missing personal contact, and the technocratic environment, however, can drastically influence the daily attention to housekeeping and the maintenance of equipment, resources, animals, and plants. Decreased personal or team motivation can lead to less focused and misplaced goal orientation. It is important to mention changes in the individual coping with the reduced privacy, increases or decreases of family contact seeking, sensation seeking, other mood changes resulting from maladaptation to confinement and isolation or the reaction to periods of high work load alternating with steep boredom. A reduced variability in self-reports and work statements are clues for the evaluation of the psychological state of crew members.

We still only have limited methods to objectively monitor all the indicators mentioned. Sensitive observation and private conversation based on a trusting personal relation between ground and crew remain the most important. Likewise, the monitoring of group cohesion and group conflict should focus on the group dynamics, cultural life events, group activities and subgroup separation (Sandal et al. 1995). Changes occurring in the group roles and functions must be noted: The possibility to develop informal leadership can disturb or support group cohesion. All interpersonal or even intercultural differences can enhance as well as hinder the group functioning. Limited resources and/or impaired competence can provoke feelings of failure or frustration, specifically anger, sense of loss, fallibility, blame, limited control, and boredom (Van Baarsen 2011), ultimately leading to depression, self-derogation, and risky behavior (Frankl 1959; Molasso 2006; Van Baarsen 2002). When unexpected competence deficits arise in individual crew members, for example, during a sudden occurrence of a life-threatening event, efficient group performance may be in danger.

For long-term missions it is nearly unavoidable that conflicts appear. The questions about why they occur and how the crew should cope with them must be answered. A rising problem is the crew autonomy of long-term missions. Long durations and long distances will heavily influence the relationship between the crew and the ground staff. Due to the distance between the earth and the space craft there will occur a delay of communication up to 20 min into one direction. That hinders, for example, an "usual" dialogue or an online support in case of trouble. It's unknown, how the crew may react if they can't see their home planet anymore ("earth out of view" effect, Kanas and Manzey 2003) or realize that they were travelling behind the "point of no return". Even Christoph Columbus would have been able to return, in principle, at each moment with his sailer "Santa Maria" if he had wanted to. This provokes a growing risk of misunderstanding, loss of belief and conflicts between the crew and ground (Kanas et al. 2007). The crew is no longer controlled by ground control, but guided, informed, and supported in decision making. Tensions in interpersonal relations affect the ability to interact with each other and increase toward the end of the mission (Cazes et al. 1996). Because of these complex conditions challenging an autonomous decision making of the crew, an independent computerized support system on board could be a great assistance not only for technical problems (Hoermann et al. 2008).

19.3 Relevance of Isolation Stress for the Cardiovascular and Immune Systems

It's known from a steady stream of recent papers about terrestrial research that loneliness in the human society (Holt-Lunstad et al. 2010; House et al. 1988; Van Baarsen 2001) is related to several potentially unhealthy changes in the cardiovascular, immune, and nervous systems (Cacioppo et al. 2002, 2010; Miller 2011). This is even more relevant for small crews with limited social alternatives. A lack of group cohesion and group conflicts changes levels and kinetics of stress hormones (Choukèr et al. 2002; Shimamaiya et al. 2004). The confinement-related limitations of activity hinder the positive effects of physical activity on immune system and mood and alter brain functions (Schneider et al. 2009, 2010). There are several findings supporting the linkage of behavioral stress and cardiovascular diseases via the immune system (Applegate et al. 1999; Cacioppo et al. 2002). That underlines the necessity of a careful monitoring also of psychological changes, e.g., the occurrence of symptoms of loneliness or asthenia. Group cohesion and group functioning is one of the most important factors, compensating or enhancing the deficits of life in isolation, promoting team 'geist' and mission motivation or evoking loneliness.

19.4 New Method Examples

As mentioned above, psychological monitoring needs new methods and technologies which are applicable, not impeding and accepted by the crew. There are first successful and promising attempts in video face emotion analysis (Dinges et al.

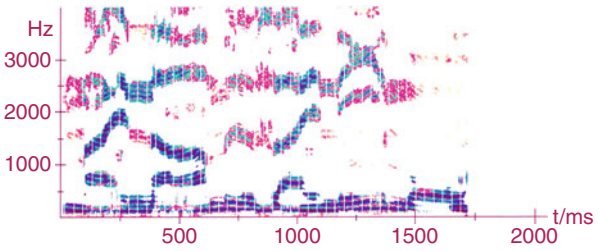

Fig. 19.1 Sonogram of a voice sample, illustrating different frequency intensities by different colors (from *blue to red*). The ordinate is given in hertz (Hz), the time abscissa in milliseconds. The lowest frequency is called fundamental frequency (voice pitch) and is related to the psychological state of the speaker. The higher frequency bands are reflections of the articulary tract and form several formants, mostly three. Formants provide relevant information for word and speaker recognition

2007) even tested already on the ISS in space. Voice frequency analysis is a very promising approach for space psychology (Johannes et al. 2000). Figure 19.1 illustrates the complex structure of voice frequencies during speech. Analyses of the human voice was first applied in space in 1965, for example, during the very first EVA by Leonov (Simonov and Frolov 1977), and continued in later MIR missions (Sulc 1979; Vaic et al. 1981). Voice pitch analysis was also verified in a long-term isolation study (SFINCSS) to indicate the actual speaker's excitation during the phone communication with the ground staff (Johannes et al. 2002). In contrast, subjective self-ratings which were assessed in parallel to the voice analyses became constant after some weeks and were of no more informative value.

The voice pitch can be registered also by means of small mobile systems and can be put together for communication analysis as illustrated for three subjects in Fig. 19.2. Such kind of voice cross analysis could not only indicate the common and individual amounts of communication but also the emotional states and reactions of the speakers.

A wireless group structure monitoring could provide information about the dynamics of the crew structure during the mission (Sandal et al. 1995). The first results of the 105-day phase of the Mars500 project promised correlations between the objectively by means of a wireless system measured time, crewmembers spend together and the classical sociogram assessment by questionnaires (Salnitski et al. 2009). Figure 19.3 illustrates the resulting group structure and its changes between different days. The average time as well as the equal distribution between crew members provides information about the crew cohesion. It will become visible if one crew member runs out of the crew and becomes isolated within isolation, which is a strong indication for psychological disturbances such as depression.

Work sample analysis (Salnitski et al. 1999, 2001, 2004) represents an already well-established method and is used as a routine monitoring. Really mission-relevant skills (e.g. the hand controlled docking of a space craft on a space station or catching a free flying object by means of a hand controlled robot arm) are refreshed and trained during the mission, thus, providing information about the proficiency of the operators and about their actual motivational state. If this professional monitoring is associated with a physiological strain assessment, an indirect

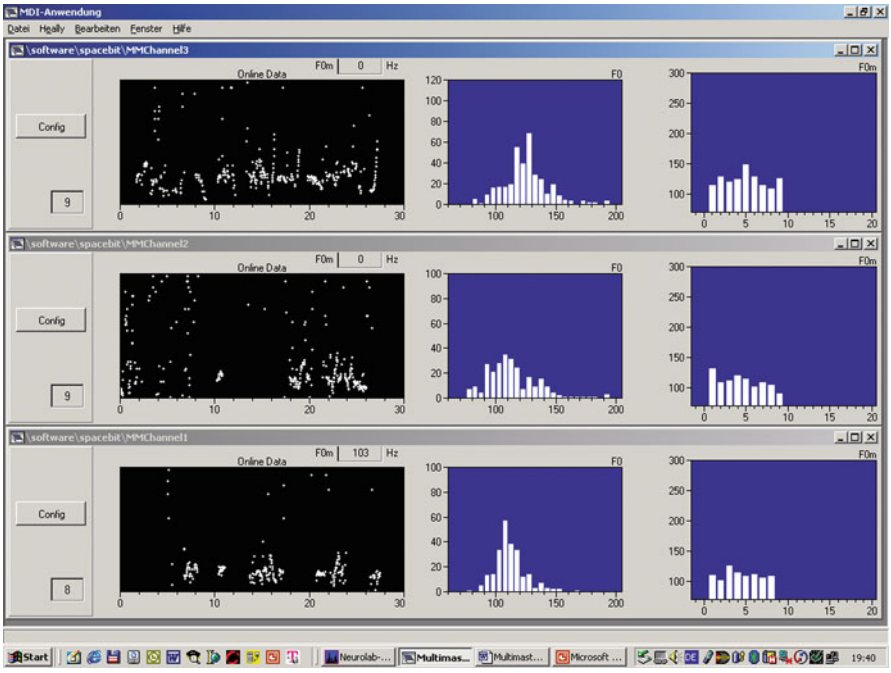

Fig. 19.2 During the (simulated) communication of three subjects their single voice pitch values are registered in 30 s intervals (*black panel*), put into histograms (*left blue panel*) and the mode of this histogram (F0m) sampled as intonation indicator and monitored over time (*right blue panel*)

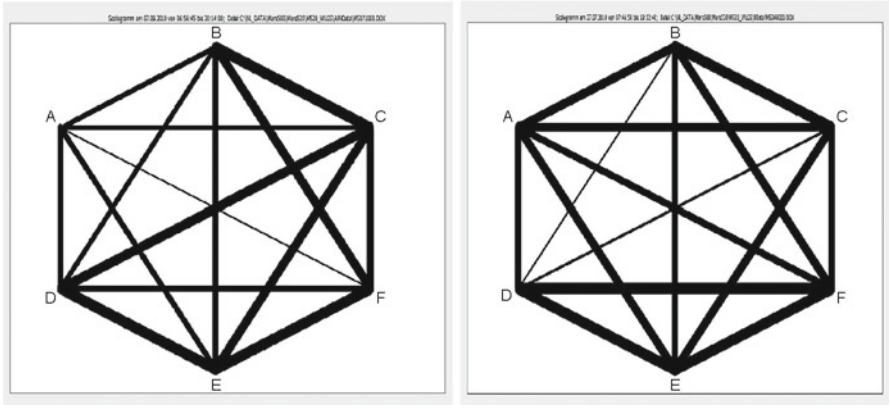

Fig. 19.3 Two examples of daily sociograms, assessed by a wireless system of small satellites, brought by each crewmember during the day, measuring the time spent together. The thickness of the lines represents the relative times spent together as a measure of personal closeness

monitoring of the psychophysiological state is provided (Johannes et al. 2001, 2003). It seems possible to implement features of embedded testing of fundamental cognitive functions into these refreshment trainers. However, areas such as

automated daily duty analysis, analysis of the cooperation quality during shared duties, analysis of readiness and progress in coping with expert-system feedback or learning new things (e.g. the language of crew mates or new skills) as well as a spare-time use analysis are currently only at the proposal level.

19.5 Summary

Because the psychological consequences of living in a hostile and potentially life-threatening environment are closely interlinked with biological responses as well, monitoring with psychological tools is an important tool to monitor stress-related neural and hormonal changes which are potentially affecting immunity and health during long-duration mission. Actually we have to state an enormous deficit of new objective methods which have to be developed and tested. However, psychological monitoring is a sensitive case. It will work under enhanced autonomy only if it provides primary support to the crew itself, and is only secondarily meant for information for the ground. If "earth" has once selected one day a Mars-mission crew, it would have to believe in it and to support it, but on the crew's demand.

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Monitoring of Autonomic Activity by Cardiovascular Variability: How to Measure?

20

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20.1 Introduction: *Why* Can Heart Rate Variability Be a Suitable Tool to Monitor Autonomic Activity?

The study of cardiovascular variability in heart rate (heart rare variability: HRV) and blood pressure (blood pressure variability: BPV) allows insight into the autonomic modulation of cardiovascular function (Malliani 2000). Most of cardiovascular variables such as heart rate, blood pressure, and stroke volume fluctuate on a beat-to-beat basis, thus creating a complex system with feedback (Aubert and Ramaekers 1999). Such a control system, well known in engineering, is more adequate to maintain stability and adapt to functional needs (Guyton et al. 1981). Therefore, cardiovascular control, as expressed by the time-dependence of hemodynamic variables, is a direct reflection of autonomic activity (Malliani 2000).

The normal heartbeat varies secondary to respiration (respiratory sinus arrhythmia) (Toska and Eriksen 1993), in response to physical (Aubert et al. 2003) and mental stress (Aubert et al. 2010) and multiple other factors; finally it is characterized by a circadian variation (Umetani et al. 1998). Both the basic heart rate and its modulation are primarily determined by alterations in autonomic activity (Cohen and Taylor 2002). Increased parasympathetic or vagal activity slows heart rate and increased sympathetic activity increases heart rate (see Chap. 6). Therefore, there is a continuous balance between the activities of both antagonist systems that regulates cardiovascular variables around a mean value. In a healthy individual, the role of the autonomic nervous system is essential to adequate cardiovascular functioning (Verheyden 2007).

However in research and hospital settings, it remained a problem how to relate these oscillations to sympathetic and parasympathetic activities. This became only

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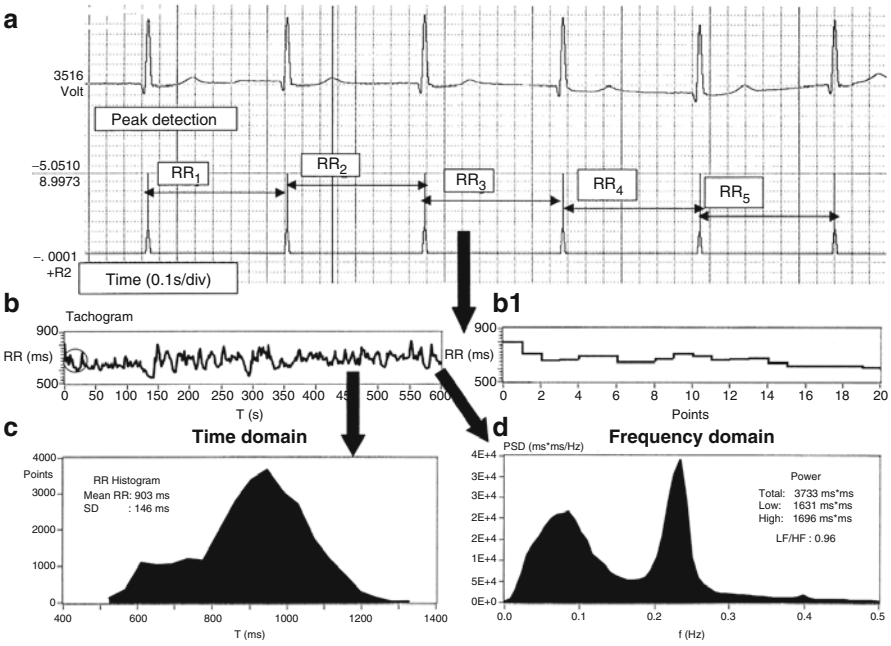

Fig. 20.1 Schematic representation of the different steps involved in HRV analysis, with courtesy from Acta Cardiologica 199:54:107107–120

possible after the advent of computer techniques in the seventies for automatic detection of fiducial points on the signals and further digital processing (Hyndman et al. 1971; Hyndman 1974; Sayers 1973). Cardiovascular changes (HRV and BPV) may be measured by a number of techniques. After peak detection on the basic parameters such as ECG and blood pressure, time series of RR intervals (tachogram) and of systolic and diastolic blood pressure values (systogram and diastogram) are obtained (Aubert et al. 1999). These signals consist of discrete time series that can be further processed either in time- or in frequency domain (Malik and Camm 1995).

20.2 Data Sampling: *What* Do You Need to Determine Cardiovascular Variability?

The first step is the recording of a high-quality ECG and blood pressure tracing. The latter is most often obtained from noninvasive recordings with a finger pulse pressure method (Imholz et al. 1998), based on the volume-clamp method of Penaz. Duration of recordings can extend from a minimum of 10 min (Task force 1996) to 24 h in Holter recordings. These signals are analog/digital converted for computer processing. In order to have a good time resolution and event definition, a sampling rate of at least 250 Hz and preferentially of 1000 Hz (giving a time resolution of 1 ms) is recommended (Pinna et al. 1994). A typical ECG signal is shown in

Fig. 20.1a. For analysis in the frequency domain, the ECG signal must meet several conditions: it must be sufficiently long (Task force 1996), random, stationary, and free of arrhythmias and noise. Random means that sequences cannot be determined by a mathematical expression or rule (Bendat and Piersol 1971). Stationary means that the probability function of the ECG signal does not change over time (Aubert et al. 1999).

The second step consists of peak detection of the QRS complex (Fig. 20.1a, lower part) or of systolic blood pressure. The result is a discrete, unevenly spaced time event series (Fig. 20.1b). Before processing, these point series have to be corrected for ectopic and missed beats. This correction is obtained by filtering and interpolation algorithms (Aubert et al. 1999).

Finally, for most spectrum analysis methods, an evenly spaced point series is needed. Therefore an evenly spaced point series is created by interpolation and a series consisting of equidistant points (0.5 s) is created.

(a) ECG recording of a young healthy subject and peak detection.
(b) Unfiltered RR-intervals depicted in a tachogram, as obtained from panel a with a time length of 600 s and insert and b1 blow-up of first 20 points of the tachogram.
(c) HRV computed in the time domain and depicted as an histogram giving the distribution of RR intervals. This histogram was obtained from a 24 h Holter recording.
(d) Analysis in the frequency domain: power spectral density (PSD), obtained from the same Holter recording. Low-frequency band: 0.04–0.15 Hz (LF); High-frequency band: 0.15–0.4 Hz (HF). Below LF there is a very low-frequency band: DC-0.04 Hz (VLF).

20.2.1 Time Domain Analysis

Parameters in the time domain are easily computed with simple statistical methods, even from short duration time windows. Their main limitation is the lack of discrimination between activities of both branches of the autonomic system (Pinna et al. 1996).

Recommendations for a standardization for different parameters have been published (Task force 1996). The most frequently used time domain parameters (Malik and Camm 1995) include SD and SDANN, both representing global variability and rMSSD and pNN50, both highly correlated to high-frequency power in the frequency domain (Ramaekers et al. 1998) and as such are also markers of vagal activity.

SDNN (or SDRR), (ms): is the standard deviation of the normal to normal (NN) interval over the recorded time interval (Copie et al. 1996; Kochiadakis et al. 1997). Theoretically, the heart rate variance which is equal to $(SDNN)^2$ is identical to the total power (ms^2) as determined from power spectral analysis (surface under the curve of PSD). It represents global HRV. SDNN depends upon the length of the recording. A longer recording of the same subject gives a higher SDNN value. Therefore it is inappropriate to compare SDNN values obtained from recordings of different durations.

SDANN (ms): is the standard deviation of the 5 min mean NN interval over the entire recording. As such this estimates changes caused by cycles longer than 5 min.

rMSSD (ms): is the square root of the mean squared successive differences between adjacent RR intervals over the entire recording. It correlates with the parasympathetic (vagal) activity as measured from HF.

pNN50 (%): is the percentage of successive interval differences greater than 50 ms computed over the entire recording.

The latter two are highly and positively correlated with each other and therefore they can be considered as surrogates for each other (Kleiger et al. 1991).

Geometric methods can be used as well to process RR-intervals. The simplest one is the sample density histogram representation (Fig. 20.1c). Another possibility consists in plotting the duration of each RR interval against the duration of the immediately preceding RR interval: Poincare maps. Assessment of HRV is based on quantification of dimension of shape of pattern.

An advantage of geometric methods is that they are very much insensitive to errors caused by ectopic beats or even arrhythmias. A disadvantage is that they are not as precise as pure time or frequency domain parameters. Also quite long-duration recordings are needed.

20.2.2 Frequency Domain Analysis

By definition, spectral analysis decomposes any steady, stationary, fluctuating signal into its sinus components. It allows plotting the power of each component as a function of its frequency (Fig. 20.1d) and the computation of the power in defined frequency regions. Power spectral analysis can be performed by nonparametric Fast Fourier Transform (FFT) (Akselrod et al. 1981) and by parametric autoregressive modeling (AR) (Baselli et al. 1985).

FFT. This approach is rather simple to apply and is computationally efficient. However its frequency resolution is limited and directly related to the duration of the recording period: the lowest frequency is the inverse of the recording length. The upper frequency limit is imposed by the Nyquist criterion: half the sampling frequency. Power spectral density can be computed in defined frequency bands: VLF, LF, and HF.

AR. In this approach the time series of RR-intervals is described by an autoregressive model: the signal at every time step is expressed as a linear function of its values at J previous steps. Therefore this method requires an a priori choice of the value of J: the order of the parametric model to provide the best fit to the data. The model order J, selected by information theory criteria, determines both centre frequency and the magnitude of the spectral components.

Spectral components and physiology (see also more extensively in Chap. 6). In a typical cardiovascular power spectrum different frequency bands can be distinguished (Fig. 20.1d): high frequency (HF: 0.15–0.4 Hz), low frequency (LF: 0.04–0.15 Hz), and very low frequency (VLF: 0–0.04 Hz) (Task force 1996). The physiological explanation of VLF has not been well defined until yet. The HF component is generally considered to be linked to respiration and thought to be mediated almost exclusively by fluctuations of efferent parasympathetic activity (Malliani

et al. 1991). HF oscillations are almost completely abolished by large dose of atropine (Akselrod et al. 1981; Ramaekers et al. 2002). However care has to be taken about respiration frequency: if that becomes lower than 0.15 Hz, it shifts to the LF component (Novak et al. 1993). The physiological background of the LF component is still rather controversial. It is believed to consist of a mixture of sympathetic and parasympathetic modulation (Eckberg 1997).

LF and HF components can be expressed in absolute units (ms^2 or $mmHg^2$), or in normalized units: LFnu and HFnu. This can be obtained by dividing the power of a given component by the total power (from which VLF has been subtracted) and multiplying by 100. As such LF and HF components are independent of total power.

Joint time-frequency analysis. Power spectrum analysis gives a global picture of oscillations present in a signal within a certain time window. Joint time-frequency analysis on the other hand can visualize dynamic or transient changes over time (Chan et al. 2001). For example, this occurs during drug infusion, tilt testing, orthostatic stress, or any time-related event. Several methods have been proposed such as: short time Fourier Transform, Choi-Williams distribution, smoothed pseudo Wigner-Ville distribution, and discrete wavelet transform (Shie and Dapang 1996).

20.2.3 Nonlinear Dynamics

Nonlinear control of heart rate and blood pressure poses potential physiological advantages in the possibility to adapt quickly and more subtly to changes in physiological needs. The basic idea behind nonlinear control mechanisms is that with a rather small amount of energy, a different end-point can be reached. Analysis methods derived from nonlinear system dynamics have opened up a new approach for studying and understanding the characteristics of cardiovascular dynamics. Nonlinear analysis methods differ from the conventional methods because they are not designed to assess the magnitude of variability but, rather, the quality, scaling, and correlation properties of the signals (Aubert et al. 2009).

However their physiological background or relationship to autonomic modulation is still a matter of debate (Beckers et al. 2006d; Dabire et al. 1998) and no up-to-date standards for nonlinear indices have been published (Task force 1996). Nonlinear indices seem rather to reflect overall, integrated control of heart rate (Lombardi 2000). Nevertheless the importance of nonlinear behavior of cardiovascular control was underlined by Yamamoto and Hughson (1991). These authors have shown that for persons in the supine (awake) position, the contribution of nonlinear fluctuations to total was $85.5 \pm 4.4\%$.

Nonlinear analysis methods can be divided into two categories: indices that describe the scaling behavior of the nonlinear system (FD, 1/f, DFAα1 and DFAα2); and indices that describe the complexity of the system (CD, LE, ApEn, NL).

Scaling: fractal dimension. A fractal object is self-similar or shows scale invariance: the details of the structure are similar when zooming in at different resolutions.

Scaling: 1/f slope. The 1/f slope of the log(power) – log(frequency) plot is obtained from the linear regression of the power spectral density from 10^{-4} to 10^{-2} Hz. A slope of -1 is an indication of scaling behavior.

Scaling: detrented fluctuation dynamics (DFA). Detrended fluctuation analysis quantifies fractal-like correlation properties of the time series and uncovers short-range and long-range correlations. The root mean square fluctuation of the integrated and detrended data is measured within observation windows of various sizes and then plotted against window size on a log–log scale. Both the short-term (4–11 beats) DFAα1 and the long-term (>11 beats) DFAα2 scaling exponents can be calculated. Values of alpha around 1 are an indication of scaling behavior.

Complexity: Correlation dimension (CD). The correlation dimension determines an order of the system i.e. the number of dimensions needed to model the dynamics of the system under consideration (Bogaert et al. 2001; Grassberger and Procaccia 1983; Raab et al. 2006). In the presence of nonlinear complex behavior, an attractor in phase space characterizes the dynamics of the system and its complexity can be quantified in terms of the properties of the attractor.

Complexity: Lyapunov exponent. The trajectories of chaotic signals in phase space follow typical patterns. Closely spaced trajectories converge and diverge exponentially, relative to each other. Lyapunov exponents measure the average rate of convergence/divergence of these neighboring trajectories. For dynamical systems, sensitivity to initial conditions is quantified by the Lyapunov exponents.

Complexity: Approximate Entropy (ApEn or SampEn). Entropy refers to system randomness, regularity, and predictability and allows systems to be classified by rate of information loss or generation.

Complexity: Numerical noise titration. The method of numerical noise titration is an analytical technique that provides a sufficient and robust numerical test to detect chaos and it gives a relative measure of chaotic intensity, even in the presence of significant noise contamination.

Nonlinear dynamics methods are a powerful addition in the tools for studying cardiovascular oscillations. Although relationship with the branches of the ANS have been shown (Mansier et al. 1996; Beckers 2002; Beckers et al. 2006a, b, d), these methods are still not widely used. Major reasons are: (1) their lack of visual representation compared to the linear methods of frequency analysis; (2) many methods are still under development and need to be validated; (3) often conflicting results are presented. Some of the conflicting findings in the literature may be due to the use of selective or nonselective blocking agents, to the use of anesthetized animals, to specific interventions (hypoxia, hemorrhage), or specific mathematical techniques. Therefore there is still a need for new well-controlled experiments, specifically aimed at the different types of receptors.

20.3 Application for Health and Disease: *When* and *Where* to Use

Cardiovascular variability has been successfully applied in innumerable domains. The purpose is not to give an overview, but just a nonlimitative list of applications: circadian variations, gender, ageing, anxiety, hostility, depression, physical training,

smoking, alcohol, caffeine, air pollution, recreational drugs, development biology, influence of gravity, myocardial infarction, sudden cardiac death, hypertension, diabetes mellitus, heart failure, heart transplantation.

Noninvasive methods are especially in favor for human spaceflight research. Therefore cardiovascular variability methods have since long enjoyed a wide popularity, as they allow a window toward autonomic modulation of the cardiovascular system.

Cardiovascular variability methods have been successfully applied in various simulation studies such as parabolic flight (Beckers et al. 2003b; Verheyden et al. 2005), head-out-of-water (Miwa et al. 1997), and head down bedrest (Pavy-Le et al. 2007). During parabolic flights short periods of about 20 s of weightlessness are obtained, between periods of 20 s of 1.8 g during acceleration and deceleration of the plane. Simulation of weightlessness during head-out-of-water and head down bedrest are both based on altering body fluid redistribution, similar to real spaceflight: In case of head-out-of-water by the hydrostatic pressure on lower limbs and for head down bedrest by tilting the bed on which the subject is lying, under an angle of 6° with the head at the lower end.

Also in Space the experience on this method is increasing and results from studies performed from the early days on and until now, especially in the IMBP Institute by the group around Baevsky (Baevsky et al. 1997, 1998, 2007) have given new insights. From their results and also from our group it could be concluded that general cardiovascular control remains stable in space (Beckers et al. 2003, 2006c, 2009; Verheyden 2007; Verheyden et al. 2007, 2009, 2010), but can be altered at the transitions between Earth to Space and back. In one occasion HRV methods were used to test the hypothesis that microgravity alters cardiovascular neural response to standardized cognitive load stimuli, induced by mathematical mental stress (Aubert et al. 2010). It was concluded that a mental arithmetic task in astronauts elicits sympathovagal shifts toward enhanced sympathetic modulation and reduced vagal modulation, in this cohort however, responsiveness was not different during microgravity. Further studies are warranted to more often monitor and for longer periods of time and in conjunction with other biological read-out parameters.

20.4 Summary

Medicine is still considered as an art by many. However, objective methods that are soundly founded and physiologically tested may turn it into a Science. Therefore further studies are warranted to more often monitor and for longer periods of time HRV and in conjunction with other biological read-out parameters. To use the HRV as one – and because of its non-invasiveness very attractive – stress monitoring tool is intriguing, on Earth and in Space. This will require very reliable hardware (ECG), data transfer and analyses, and a solid database to allow for conclusive interpretation hereby providing adequate recommendations for respective countermeasures or further diagnosis of dysfunctional organs which are affected by imbalanced autonomic regulation, like the immune system.

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Breath Gas Analysis

21

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21.1 Introduction

Since ancient times human breath has been used in assisting to diagnose diseases like diabetic coma, liver diseases, and renal failure. Similar to the exchange of carbon dioxide and oxygen by the lungs numerous volatile substances either endogenously produced or exogenously absorbed are exchanged via the lungs. However, despite the knowledge on the diagnostic properties of exhaled breath, the smell underlying substances in nature remained undiscovered until recently. A first step toward a better understanding was done in 1971 when Pauling detected about 250 substances in the exhaled breath of humans (Pauling et al. 1971). Thereafter, the implementation of increasingly sensitive diagnostic platforms since the 1980s has led to the identification of several chemical compounds within human exhaled breath. A sample of examples of so-far identified volatile organic and inorganic compounds (VOICs) in human exhaled breath together with a possible clinical application is given in Table 21.1.

21.2 Technical Approaches for Breath Analyses

As compared to most other diagnostic methods used in medicine, the analysis of exhaled breath is truly noninvasive and allows an unlimited number of repetitions. Furthermore, "breath gas analysis" is not only restricted to the analysis of breath. The same analytic method could be applied to gaseous headspace probes over any liquid originating from the human body (Pinggera et al. 2005). Currently operated diagnostic platforms for the analysis of exhaled breath and headspace VOIC

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Table 21.1 Selected examples of exhaled breath compounds, potential origin, and potential clinical application

Compound	Source	Potential clinical application
Acetaldehyde (Moeskops et al. 2006; Smith et al. 2002)	Ethanol metabolism Oxidative stress	Monitoring of ethanol metabolism and oxidative stress
Acetone (Turner et al. 2006a)	Decarboxylation of acetoacetate Dehydrogenation of iso-propanol	Monitoring of diabetes
Acetonitrile (Buszewski et al. 2009; Pinggera et al. 2005)	Uptake from cigarette smoke	Monitoring of smoking behavior
Alkanes and monomethylalkanes (Phillips et al. 2004)	Lipid peroxidation	Heart allograft rejection
Ammonia (Davies et al. 1997; Endre et al. 2011)	Protein metabolism	End stage renal disease
Dimethyl sulfide (Van den Velde et al. 2008)	Liver failure	Monitoring of liver damage
Ethane (Kazui et al. 1994; Risby and Sehnert 1999; Stenseth et al. 2007)	Lipid peroxidation product	Oxidative stress monitoring
Ethanol (Kramer et al. 2007; Smith et al. 2002)	Gut bacteria, external uptake	Alcohol intoxication
Ethylene (Risby 2005)	Lipid peroxidaton product	Oxidative stress monitoring
$^{13}CO_2$ (Modak 2007; Graham et al. 1987)	Metabolism	*Helicobacter pylori* breath test, gastric emptying, gut disease, liver function, pharmacokinetic monitoring
Hydrogen (Christl et al. 1992; Bond and Levitt 1976)	Gut bacteria	Lactose malabsorption breath test
Isoprene (Karl et al. 2001; Turner et al. 2006b)	Cholesterol biosynthesis	Statine therapy monitoring
Malondialdehyde (Scholpp et al. 2002)	Lipid peroxidation product	Oxidative stress monitoring
Methane (Roccarina et al. 2010)	Gut bacteria	Gastrointestinal diseases
Nitric oxygen (Kövesi et al. 2003; Pedrosa et al. 2010; Turner 2007; Karlsson et al. 2009)	Nitric oxide synthetase	Monitoring of airway inflammation and ischemia-reperfusion injury
Propionaldehyde (Moeskops et al. 2006; Steeghs et al. 2006)	Lipid peroxidation product Propanol metabolism	Oxidative stress monitoring
Propofol (Hornuss et al. 2007)	Intravenous anesthetic	Monitoring of pharmacon during anesthesia
Trimethylamine (Endre et al. 2011)	Uptake of trimethylamine or precursor	Monitoring of hemodialysis efficacy
Pentane (Li et al. 2009; Schubert et al. 1998, 2005)	Lipid peroxidation product	Monitoring of oxidative stress

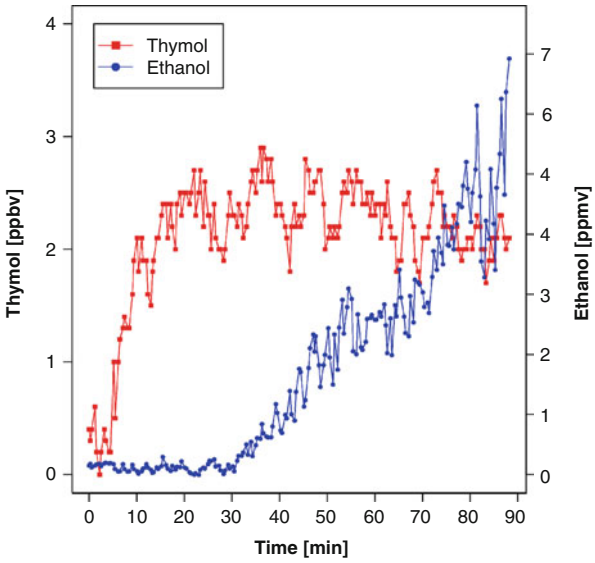

Fig. 21.1 Example for the on-line measurement of exhaled breath concentrations of thymol and ethanol after oral ingestion of cough syrup containing thyme and 7 vol.% ethanol as solvent by the ion molecule reaction mass spectrometry system

composition include gas chromatographic (GC) and direct mass spectrometric methods. Depending on the analytical system and the respectively used setups, sensitivities down to the parts per trillion per volume level are reported for both methods (Amann et al. 2007).

Recent developments lead to an increase in sensitivity and decreases in time requirements in GC methods. Furthermore, coupling of GC with mass spectrometric methods (GC-MS) improved rapid compound identification. However, the disadvantages associated with the use of GC methods are the ongoing need for preconcentration, substance calibration, and limited capability for on-line measurements. Nevertheless, if definite compound identification is warranted GC still represents the reference method. Among direct mass spectrometry methods the following systems have been used recently for breath gas analysis: (a) ion molecule reaction mass spectrometry (IMR-MS); (b) ion mobility spectroscopy (IMS); (c) proton transfer reaction mass spectrometry (PTR-MS); (d) and selected ion flow tube mass spectrometry (SIFT-MS). The major advantage of all these instruments is that preconcentration procedures are unnecessary, which allows their possible use for breath-by-breath on-line measurements of the exhaled breath VOIC composition. For IMR-MS even the on-line measurement in volunteers (Fig. 21.1), critically ill patients (Fig. 21.2), and in patients undergoing cranial surgery has been shown (Lindinger et al. 1998; Smith et al. 2002; Dolch et al. 2008; Hornuss et al. 2007).

21.3 Applications and Results

Clinical breath gas analyses is an emerging tool offering unique diagnostic capabilities for a large number of disorders which include bacterial infection (Braden 2009), metabolic disorders (Romagnuolo et al. 2002), pulmonary disease (Turner 2007),

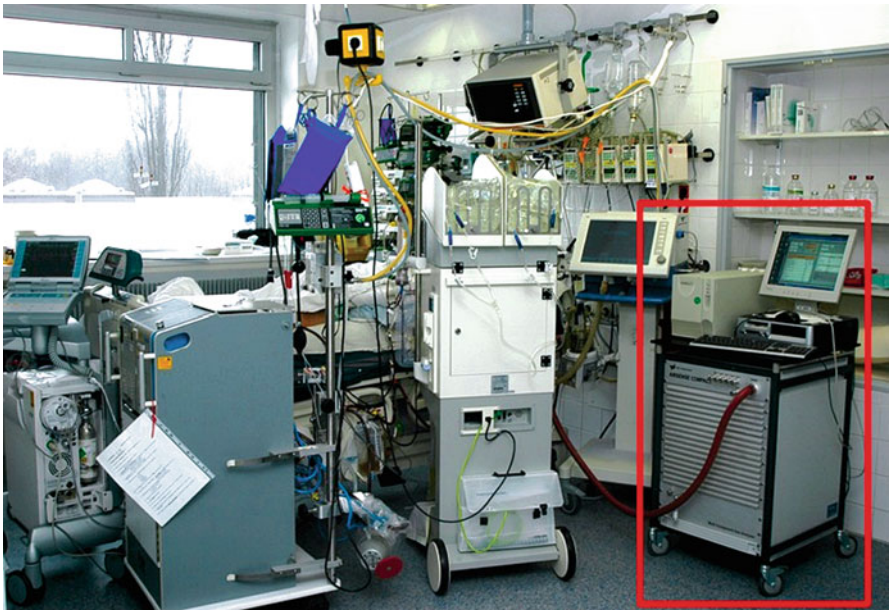

Fig. 21.2 Ion molecule reaction mass spectrometer (IMR-MS, *red box*) during on-line monitoring of exhaled breath in a patient with acute respiratory distress syndrome (ARDS). A directly to the patients endotracheal tube connected steel T-piece allows gas transfer from the patient to the IMR-MS (*red rubber tubing*). The vacuum pumps define the size of the hardware. In space, technical solutions might use the external vacuum instead

and cancer (Szulejko et al. 2010). However, potential biomarkers for these disorders are part of a complex matrix of different VOICs present in human breath and the understanding of their pathophysiological role is still in its infancy. This is also due to i) the high number of VOICs reported in human breath ranging from <150 (Gordon et al. 1985) to 3,500 (Phillips et al. 1999), ii) the instrumentation and techniques used for breath analyses (Buszewski et al. 2007) and iii) on the choice of physical methods for capturing volatile compounds in exhaled breath and sample preparation and preconcentration (Szulejko et al. 2010). Apart from technical and analytic aspects, the large variability in the number and concentrations of exhaled VOICs reported by different researchers has a number of other possible causes which include (a) subject demographics, (b) oral hygiene, (c) diet, (d) ambient air contaminants, and (e) the portion of expiratory air which was sampled. Therefore, further research is urgently needed to evaluate the impact of these factors on exhaled breath measurement results.

The impressive progress in breath gas analysis during the last decades has already resulted in some realized applications in medicine. So far the measurement of ethanol, carbon dioxide (CO_2, ventilation), $^{13}CO_2$ (*Helicobacter pylori* breath test, pharmacokinetic monitoring), hydrogen (H_2, lactose malabsorption breath test), nitrous oxide (N_2O, anesthetic agent), nitric oxygen (NO, asthma, airway

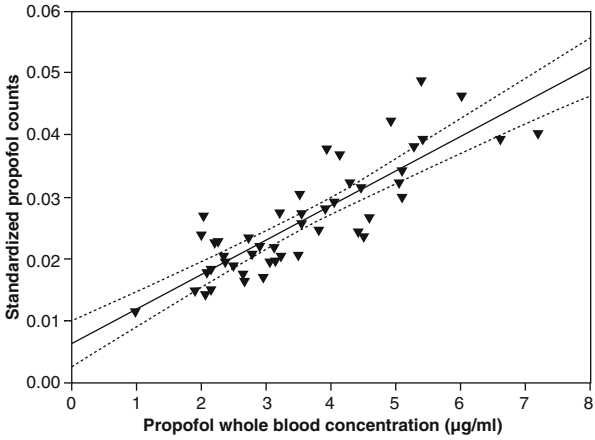

Fig. 21.3 Correlation of human propofol whole blood concentration and expiratory air propofol measured by ion molecule reaction mass spectrometry during surgery. (From Hornuss et al. 2007)

inflammation), and volatile anesthetics in exhaled breath has achieved FDA approval for clinical use (U.S. Food and Drug Administration, 2011). Furthermore the pharmacokinetic monitoring of propofol, a widely used intravenous anesthetic, in exhaled breath has come within technical reach (Fig. 21.3) (Hornuss et al. 2007) and hand held devices have been developed for the measurement of CO_2, ethanol, and NO. As the result of ongoing research activity the number of VOICs with a probable stress and disease association identified is constantly increasing. Other metabolic markers, like acetone, as one of the most abundant VOICs in human exhaled breath, are closely linked to dextrose metabolism and lipolysis. Increased concentrations of ketone bodies in blood are present in patients with uncontrolled diabetes or during starvation as the result of acetyl-CoA decarboxylation (Miekisch et al. 2004; Schmoelz et al. 2007). Isoprene, one of the main hydrocarbons endogenously produced in mammals (Gelmont et al. 1981) is closely linked to cholesterol biosynthesis. Typically concentrations around 100 parts per billion of volume (ppbv) are present in the exhaled breath of adults (Karl et al. 2001; Turner et al. 2006b) and statin therapy caused a decrease in exhaled breath isoprene (Karl et al. 2001). Acetonitrile, a saturated aliphatic nitrile, for example, is absorbed from cigarette smoke and present within exhaled breath and urine headspace samples solely in smokers (Pinggera et al. 2005; Buszewski et al. 2009). Ammonia and trimethylamine were found to be increased in patients with end-stage renal failure and are possibly useful for the monitoring of dialysis efficacy (Davies et al. 1997; Endre et al. 2011). Dimethysulfide was found to be increased in patients with liver failure and accounts to the sweet and musty smell (Van den Velde et al. 2008). Several compounds present in human breath have been identified as end products of oxidative stress-mediated lipid peroxidation. Acetone, ethane, malondialdehyde, pentane, and propionaldehyde are increased in inflammatory processes as ischemia reperfusion injury (Miekisch et al. 2004; Li et al. 2009; Risby and Sehnert 1999) or "sterile" gravitational stress-dependent immune activation during parabolic flight (Choukèr et al. 2007), acute respiratory stress syndrome (Scholpp

et al. 2002), and ultraviolet light-induced lipid peroxidation (Steeghs et al. 2006). Furthermore, NO, an important cell-signalling molecule that can be released in pathological amounts by the NO synthase during inflammation, is increased during asthma exacerbation (Pedrosa et al. 2010; Turner 2007) and lung transplant reperfusion injury (Kövesi et al. 2003).

21.4 Future Perspectives for Space Applications

Manned spaceflights represent an extreme example of exposure to stressful environmental challenges and monitoring of crew health status represents unique technical and medical challenges. Especially in the light of future long-duration manned Lunar and Martian exploration or outer space missions, the monitoring of health is a prerequisite for mission accomplishment. Here, the analysis of biomarkers in expiratory air offers promising noninvasive diagnostic options to monitor human health status because blood sample return to earth will not be possible and on-site monitoring can be limited. Moreover, this technology represents a very promising technique for dual analyses of volatile masses in the exhaled air as well as in the spacecraft or habitat environment, respectively. As described above, some important biochemical pathways have been identified as the source of VOICs that are released from tissue into the gas phase indicating metabolic changes, immune activity, and oxidative stress. Moreover, this technology can be also of important value for pharmacokinetic monitoring when drugs are used and can be of high value to assess the compliance of taking the prescribed drugs (Hornuss et al. 2007). To better and further understand the read-out parameter of exhaled air analyses also in the light of space applications, this technique has been implemented now in multiple scientific investigations, to understand the influence and the impact of stressor in space, like weightlessness (see Fig 21.4), confinement, nutritional changes, hypoxia, and radiation exposure and to compare those to standardized baseline values gathered under full environmental control. The following studies have been conducted with our partner institutions to monitor human adaption to space-relevant conditions of life, accordingly: “CHOICE” study (Antarctica, hypoxia and isolation, see also Chaps. 13 and 31), the “MARS500” project (long-term isolation, control for multiple stressors, nutritional modification), short and mid-term bedrest (immobilization, test for artificial gravity or nutritional countermeasures), or the NEEMO (hyperbaric/-oxic stress and simulation of extravehicular activities (EVA, “spacewalks”)) in the NASA aqueous habitat. In space, first investigations on exhaled volatile compound were successful to monitor exhaled breath NO in crew members of the international space station (Karlsson et al. 2009). This approach will be further extended to establish VOIC values suitable for monitoring health alterations as well as general homeostasis, allostasis, or allostatic load (see Chap. 3) from expiratory air collected from the crew of the ISS. This upcoming, multinational and multidisciplinary study is named CONSCIOUS (Consequences of Stress Challenges on stress response systems and Immunity in Space).

Fig. 21.4 Blood and air sampling (in vials) from two healthy subjects during a parabolic flight mission, in front: operator. With permission

21.5 Summary

Breath gas analysis is an emerging technology that may open the opportunity to intensify longitudinal health monitoring, during space exploration, and to provide a noninvasive research tool for the determination of organ functional changes when subjected to stressors in space, which includes also the effects of countermeasure (e.g. nutritional, exercise, pharmacologic) hereto. Also, the dual use property can allow for the assessment of environmental changes in the habitat with respect to air composition and pollution. Results of either application can be evaluated on-site or transferred electronically to earth for further interpretation. However, further in-depth investigations are warranted to better understand the results provided "breath-by-breath". Hereby, research for applications in patients as well as for space crews are as described fully complementary and cofertilizing and both will benefit from this technology in the future.

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Monitoring the Microbial Burden in Manned Space Stations

22

Rob Van Houdt and Natalie Leys

22.1 Introduction

Astronauts on mission on the International Space Station (ISS) experience the uniqueness of this space station in all its aspects. The high technological small habitat confines alternating crews ranging from 3 to 10 members, protecting them from the extreme space environment (pressure, temperature, radiation) and providing them the necessary supplies (air, water, food). While living in space and inside sophisticated space vehicles, astronauts face unique stressors that they did not encounter before on Earth and its natural environment.

Microbial contamination is a stress factor per se and can be of health relevance in the condition of an impaired immunity. In addition, immune alterations are elicited and amplified by microgravity and/or other stress conditions in space (see chapters in Part III of this volume) hereby further increasing the vulnerability to infections. As being confined in space, not surprisingly, such a man-made environment will generate its own unique microbial population, which mainly originates from the crew (skin, upper respiratory tract, mouth, and gastrointestinal tract) but also includes environmental microorganisms. The diversity and mass of the microbial population is also specifically shaped partly due to the restricted hygienic practices in space (e.g., reduced gravity and water limitations make showers impossible).

Most of these microorganisms (both environmental and from human origin) do not present severe hazards for healthy people; however, they can pose threats to astronauts with reduced immune response. Therefore, the total load and diversity of environmental microorganisms needs to be controlled, to guarantee adequate living quality and reduce the risks of harmful effects on the crew. Especially, the (opportunistic)

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Table 22.1 Environmental microbial quality standards for air, surfaces, and water in ISS

	Maximum for bacteria	Maximum for fungi
Pre-flight		
Air	300 CFU/m^3	50 CFU/m^3
Internal surfaces	500 CFU/100 cm^2	10 CFU/100 cm^2
Potable water[a]	50 CFU/mL	ND
In-flight		
Air	1,000 CFU/m^3	100 CFU/m^3
Internal surfaces	10,000 CFU/100 cm^2	100 CFU/100 cm^2
Potable water[a]	50 CFU/mL	ND

ND not determined, *CFU* colony forming unit
[a]Coliforms may not be detected

pathogens – capable of causing infections and disease – are undesired and their manifestation, both in numbers and diversity, needs to be prevented.

Thus, in an effort to reduce the health hazards posed by microbial contamination, international quality standards for air, surfaces, and water have been defined and prevention, monitoring, and mitigation measures have been implemented by the space agencies.

22.2 International Environmental Quality Standards for the ISS

To ensure the indoor environmental quality in space stations, the maximal amounts of bacteria and fungi in air, on surfaces, and in water permitted in the living and working areas of the ISS were internationally defined and agreed upon. These quality limits were included in the ISS Medical Operations Requirements Document (Duncan et al. 2008; ISS MORD 2009). Thresholds were mainly based on minimal concentration levels that are "achievable" with the current prevention and monitoring technologies available and applicable for space (Table 22.1). These thresholds are defined as the maximum total number of aerobic and heterotrophic viable cells of bacteria or fungi, counted as colony forming units on a rich agar medium.

These internationally defined standards for in-flight air quality in ISS are comparable to the threshold limits frequently used for healthy offices (Sessa et al. 2002; Dacarro et al. 2003) or, more in general, to assess indoor air quality in Europe (Brief and Bernath 1988). These ISS levels can be placed approximately in the category intermediate for bacteria and low for fungi based on the classification of the European Collaborative Action "Indoor Air Quality and its Impact on Man" for bacteria and fungi in homes and offices (CEC 1993). Likewise, ISS drinking water standards are comparable with Earthly US standards (Dawson and Sartory 2000) and World Health Organization guidelines (WHO 2008). For surfaces on Earth, no general guidelines exist since requirements for surface cleanliness depend on the activity (e.g., domestic, industrial, food handling, hospitals, and others).

For preventing microbial distribution through air and to assure the microbial burden remains below the thresholds at all times, the air in the ISS is filtered.

Table 22.2 Microbiological testing procedure for noncommercially sterilized foods

Test	Limit for rejection
Total aerobic count	If > 20,000 CFU/g in a sample or If > 10,000 CFU/g in more than 1 sample
Yeast and molds	If > 1,000 CFU/g in a sample or If > 100 CFU/g in more than 1 sample
Coliform	If > 100 CFU/g in a sample or If > 10 CFU/g in more than 1 sample
Coagulase-positive *staphylococci*	If > 100 CFU/g in a sample or If > 10 CFU/g in more than 1 sample
Salmonella	If > 0 CFU/g in a sample

CFU colony forming unit

The Russian Segment (Service Module, Functional Cargo Block FGB, and Mini-Research Modules 1 & 2) uses pleated woven filters. In the U.S. Segment of ISS (Nodes 1, 2, and 3, Lab, Airlock, Japanese Experiment module JEM-PM, and Columbus) air is filtered through High Efficiency (HEPA) filters (Duane L. Pierson, pers. comm.). Both provide fine aerosols filtration (from 0.3 μm) and strongly reduce the total amount of dust particles in the air, and the microbes associated with that. Thus, filters continuously collect, but do not inactivate microbes from the ISS air and are replaced when saturated. In addition, ISS has two POTOK 150MK Russian air filtration and disinfection systems, one in the Zvezda module and a second one in the Zarya module. POTOK 150MK provides microbe inactivation with electrostatic pulses and charged ions, followed by fine aerosols filtration with an efficiency up to 99% for particle sizes ranging from 0.01 to 10 μm (Volodina et al. 2003). Hardware and spacecraft interior surfaces are treated pre-flight with heat, radiation, or chemicals, to reduce the microbial burden to the minimum (Debus 2006). To prevent microbial contamination in the potable water, iodine (2–5 mg/L) or silver (0.5 mg/L) is added pre- and in-flight to the water supplies in Shuttle or ISS.

Food production, packaging, and products are strictly controlled and tested for microbial contamination pre-flight to ensure that they are in compliance with microbial standards for spaceflight foods (NASA 2006; Perchonok and Douglas 2008; ISS MORD 2009). Actually, Hazard Analysis Critical Control Point (HACCP), which is a systematic preventive approach assuring the safe production of foods, was developed by the Pillsbury Company while working on producing foods for NASA for spaceflights in the early 1960s (Lachance 1997). These analyses include for noncommercially sterilized foods both the raw materials (before flight packaging), which are tested for specified microorganisms depending on the product (five samples from each lot), as well as the finished goods (flight packaged), which are tested for total aerobic count (one sample from each daily production) (Table 22.2) (NASA 2006).

In addition, in-flight monitoring strategies have been defined and implemented to systematically evaluate these quality standards (ISS MORD 2009). Surface and air samples are collected regularly (once every 90 days for the U.S. segment) from different sites of the U.S. and Russian segment with the U.S.-supplied Surface Sampler

Kit (SSK) (NASA 2005a) and Microbial Air Sampler (MAS) Kit (NASA 2005c) and the Russian-supplied Sample Tube Kit and Ecosphera Kit (Novikova 2004), respectively (ISS MORD 2009). The quality of potable water from different ports in the Russian (SRV-K, SVO-ZV, CWC see below in section Water) and the U.S. segment (WRS) are checked once every 3 months and each month, respectively. The samples are processed and analyzed in-flight with the U.S.-supplied Water Microbiology Kit (NASA 2005b; ISS MORD 2009). In addition, samples are regularly archived for further analysis postflight. The sampling schedule and frequency is adjusted when recommended by the U.S. and Russian experts to ensure water quality aboard the ISS.

22.3 The Environmental Microflora in the ISS

22.3.1 Air

Airborne microbial contamination can have diverse health effects: it can cause irritation, respiratory infections, and allergic diseases (Husman 1996). Microbes can become airborne via diverse routes like talking, coughing, sneezing, and sewage removal. Dispersion and deposition are directed by general physical principles with droplet size being one of the most important factors (Morawska 2006), and dust particles play an important role as carriers. In addition, humidity, oxygen concentration and time affect the stability of airborne microorganisms and their survival in droplets or dust particles (Cox 1995). In space, dust and microbes do not sediment in microgravity which results in persisting airborne aerosols and high microbial densities in cabin air, particularly if the cabin air filtering systems are not well maintained. The design and operational characteristics of a confined space station has also an impact on the spread of (bio) aerosols (Castro et al. 2006).

The airborne bacterial and fungal contamination levels were previously also monitored during the occupation of the Mir station (1986–2001). While the bacterial population was fairly stable with 95% of the samples below 500 CFU/m^3 of air, the fungal contamination varied considerably from 2 up to 10,000 CFU/m^3 (Novikova 2004). A similar survey performed aboard ISS (from the year 1998 to 2005) indicated that both the airborne bacterial and fungal contamination were low with a maximum of 710 and 44 CFU/m^3, respectively (Novikova et al. 2006) (Table 22.3), which is reflected in the current ISS limits of 1000 bacterial and 100 fungal CFU/m^3. The lower contamination levels in the ISS compared to Mir were primarily due to the installation of the efficient Russian air disinfection and filtration system POTOK 150MK (POTOK Inter, Moscow, Russia) in April 2001. A similar decrease in contamination levels was also observed in the Mir station after installation of this system in January 1998 (Novikova 2004). The latter illustrates that rational habitat design is essential and probably most cost-effective in the long term.

Although the airborne microbial contamination levels were generally below the limits (see above), these overall levels do not necessarily reflect the real associated risks since they do not provide information about the pathogenic potential of the

Table 22.3 Environmental microbial contamination reported for air, surfaces and water in ISS

In-flight	Bacteria	Fungi	Reference
Air	<710 CFU/m^3 Dominant: *Staphylococcus* and *Bacillus* spp.	<44 CFU/m^3 Dominant: *Penicillum* and *Aspergillus* spp.	Novikova et al. (2006)
Internal surfaces	24–43,000 CFU/m^2 Dominant: *Staphylococcus* and *Bacillus* spp.	25–300,000 CFU/m^2 Dominant: *Penicillum* and *Aspergillus* spp.	Novikova et al. (2006)
Potable water	Often >100 CFU/100 mL Dominant: *Methylobacterium, Ralstonia, Sphingomonas* and *Pseudomonas* spp.	ND	Bruce et al. (2005)

ND not determined, *CFU* colony forming unit

contaminants. Therefore, it is valuable to include identification assays to detail the predominant microbial taxa. *Staphylococcus* and *Bacillus* spp. were found to be the dominant bacterial species in the air aboard ISS (Castro et al. 2004), and similar data were also obtained in surveys in other non-space-related confined environments, such as airplanes (Osman et al. 2008) and polar stations (Van Houdt et al. 2009a). In particular *Staphylococcus aureus* was observed frequently (in 3.2% of the cases), and exchange of *S. aureus* strains among crew members was already observed in previous missions (Berry 1973). *S. aureus* colonizes naturally the skin or nose of healthy people, but can also cause a range of illnesses. The dominant fungal species were *Penicillum* and *Aspergillus*, with *Aspergillus flavus* most frequently present (observed in 2.5% of the cases) (Novikova et al. 2006).

22.3.2 Surface

Direct contact is another major route of transmission of microorganisms. Most microorganisms are able to stick to surfaces and form biofilms. It is well documented that this process of biofilm formation has major implications for many industrial and health-related processes (Van Houdt and Michiels 2010).

A large ISS monitoring campaign (from 1998 to 2005), including 243 surface swab samples, indicated that the bacterial and fungal contamination ranged from 25 to 43,000/100 cm^2 and from 25 to 300,000/100 cm^2, respectively (Novikova et al. 2006) (Table 22.3). Although the levels fluctuated within a broad range, surface contamination levels were in most cases low and below the international quality limit (i.e., 10,000 bacterial and 100 fungal CFU/100 cm^2). The dominant bacterial and fungal species isolated were similar to those present in the airborne contamination, thus revealing predominant *Staphylococcus* and *Bacillus* bacterial species, and *Penicillum* and *Aspergillus* (e.g., *A. niger* [see Chap. 10]) fungal species. Occasional increases in bacterial and fungal contamination were registered for some locations, such as on a panel of the ventilation screen and the table surface in the Service Module or behind panels of the Functional Cargo Blok (Novikova et al. 2006; James

et al. 2008). Disinfectant wipes were used each time contamination levels were above the quality threshold and resulted systematically in a decrease of contamination below the acceptability limits. These disinfection wipes contain either a quaternary ammonium compound (supplied by U.S.) or a mixture of hydrogen peroxide and a quaternary ammonium compound (supplied by Russia). Nevertheless, the resilience of contamination at certain locations could indicate either a resistance to the used disinfectants, favorable growth conditions (e.g., humidity), or both.

22.3.3 Water

Microbiological contamination in drinking water is a well-known threat, not only from health perspective but also for microbial-mediated corrosion (Szewzyk et al. 2000; Berry et al. 2006). One of the important microbial characteristics in drinking water storage and distribution systems is biofilm development, which increases the persistence of pathogens and the resistance to disinfectants (Stewart et al. 2001; Emtiazi et al. 2004; Wingender and Flemming 2004; Van Houdt and Michiels 2010). This increased resistance can be attributed to different mechanisms such as a slow or incomplete penetration of the biocide into the biofilm, an altered physiology of the biofilm cells, expression of an adaptive stress response by some cells, or differentiation of a small subpopulation of cells into persister cells (Van Houdt and Michiels 2010). Adequate monitoring and disinfection methods are therefore needed to mitigate the risk to hardware disintegration and crew health.

Different sources provide the ISS with drinkable water. Ground-supplied water has been provided by the Russian PROGRESS, European ATV, and American Shuttle vehicles (Bruce et al. 2005; James et al. 2008; Straub et al. 2009), respectively. It is expensive to ferry water from Earth to space, with transport costs to deliver items to the ISS running up to 10,000 Euros per kilogram (Jenkins 1996), and thus water has to be recycled in space. During Shuttle flight, potable water is collected from the fuel cells in Contingency Water Containers (CWC) and transferred to ISS upon arrival (Bruce et al. 2005; James et al. 2008; Straub et al. 2009). Inside ISS, humidity condensate (mainly from the crew's breath and sweat) is collected and purified by the Russian SRV-K system located in the Service Module (Bruce et al. 2005). Since May 2009, the U.S. Water Recovery System (WRS) in the U.S. Segment creates potable water by recycling humidity condensate and urine distillate, which is then distributed by the Potable Water Dispenser.

The ISS SVO-ZV system, which dispenses at ambient temperature both the Russian ground-supplied and the CWC water for consumption, was monitored 27 times over a period of 4 years (2001–2004). In-flight analysis indicated that in 16 cases (60%) the bacterial levels were above the acceptability limit of 100 CFU/100 mL (Bruce et al. 2005). Most of the isolates recovered from these samples were typical waterborne gram-negative bacteria from the genera *Methylobacterium*, *Ralstonia*, *Sphingomonas*, and *Pseudomonas*. Although this contamination does not pose an immediate threat to the astronauts (only some species are recognized as opportunistic pathogens), high microbial concentrations aboard spacecraft prevents consumption

of the water as currently no identification method is available during flight. Therefore, this type of contamination wastes large amounts of crew time and Earth-based resources. In addition, these water contamination events require more materials to be transferred both to and from the ISS, which is very costly.

This recurrent water contamination elicited a series of remediation actions, however, contamination levels increased again above the limit soon afterward (Bruce et al. 2005). This resilience of the contamination could be caused by different events. The bacterial population could have adapted to survive in such water systems with the formation of a starved and nonculturable but metabolic active fraction of the population, which is more resistant and capable of (cryptic) growth on the biocide-killed fraction. Survival in ultrapure water for up to 6 months and in river water up to 4 years has indeed been described for a *Ralstonia* sp. isolated from ultrapure water systems (McAlister et al. 2002) and *Ralstonia solanacearum* (Alvarez et al. 2008), respectively. In addition, the bacterial population could harbor resistance mechanisms toward silver, which is used as biocide in the ISS potable water (Straub et al. 2009). *Cupriavidus metallidurans* strain CH34 (formerly *R. metallidurans* CH34) has been studied intensively for its resistance toward multiple heavy metals among which is silver (Janssen et al. 2010). Most of these mechanisms are located on large plasmids (Monchy et al. 2007; Janssen et al. 2010), but some are also located on mobile genetic elements integrated in the chromosome (Van Houdt et al. 2009b) or on the chromosome itself (Janssen et al. 2010). The presence of similar plasmids have been described in isolates from ISS water (Mergeay et al. 2009), indicating that they are putatively equipped with silver resistance mechanisms. Finally, certain areas in the water system could be prone to harbor and build up bacterial biofilms, which could augment the above described aspects.

22.4 Conclusions

The collected data of microbiological contamination from different environmental sources indicates that the ISS, which is in-flight for almost 10 years, is a microbiologically safe working and living habitat. Nonetheless, occasional contamination hazard reports do indicate that the current prevention and monitoring strategies are a minimum to control the microbial burden in manned space stations. Fluctuations in microbial concentrations and contamination events suggest the need for continued diligence and evaluation, as well as further improvements in engineering systems.

Prevention is an essential component in (future) orbital and planetary space stations and design should integrate, next to thermal, mechanical and chemical resistance of equipment and utensils, also the hygienic properties (e.g., biofouling or antimicrobial surface properties). Thus, rational habitat design, integrating both these constructional and health-related parameters, is essential and probably most cost-effective in the long term. Specific for the spread of biological aerosols, the development of a reliable model is important to pinpoint critical locations in a certain habitat design.

In addition, monitoring tools could be optimized by developing online detection tools that can be used directly in flight and that are capable of simultaneous quantification and identification. These molecular, nonculture-dependent assays would be less time consuming for the astronauts and circumvent the necessity for postflight analyses, allowing quick and autonomous decisions by the crew for assessment and remediation of contamination problems in the air, on surfaces, or in water and food.

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Monitoring of Body Core Temperature in Humans

23

Andreas Werner and Hanns-Christian Gunga

23.1 Introduction to the Assessment of Thermoregulation in Man

Humans have an endothermic metabolism and the autonomous regulation mechanisms ensure that the body core temperature for the vital organs in the body (brain, heart, liver, and kidney) is kept around 36.7°C in the core with circadian variations of ±0.5°C in males. In females the menstruational cycle alters the body core temperature in addition. Scientific investigations to monitor thermoregulation by the variations of core temperature, are usually performed by placing a thermosensor in the esophagus, nasopharynx, rectum, or tympanum/auditory meatus. However, none of these methods are neither really applicable nor convenient during daily routines, especially during long-term recordings for chronobiological research. This is due to the fact that the requirements for a method serving to measure body core temperature are demanding; the thermosensor has to be noninvasive, be easy to handle, must fulfill basic hygiene standards, not be biased toward various environmental conditions. Moreover, the quantitative changes should appropriately reflect small deltas of the arterial blood temperature changes and the response time of the thermosensor to temperature changes should be as short as possible (Cooper et al. 1964; Gundel et al. 1997). These requirements are essential because several studies in humans have shown that if high environmental temperature and humidity prevail (Kirsch et al. 1996, 1999; Pandolf et al. 1988) – especially in combination with heavy physical workloads and fluid loss (sweating) with inadequate re-hydration (Gunga et al. 1991) – the heat load will lead to a rapid rise in the body core temperature, subsequently resulting in heat stress-related injuries such as heat stroke (Gunga 2008; Gunga et al. 1993, 2008b; Kirsch and Vogt-Kirsch 1985; Montain et al. 2001; Sawka and Wenger 1988; Taylor 1998). If thermoregulatory

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impairments due to fever or drugs prevail, the deleterious developments may occur even faster (Gunga et al. 2005; Hoyt and Friedl 2004; Hoyt et al. 2002; Clark and Lipton 1984; Imrie and Hall 1990).

The measurement of the circadian rhythm of the body core temperature in man requires a continuous data recording. Up to now, the common sensors to measure the valid body core temperature are either semi-invasive (e.g., rectal, oesophageal or urine bladder probes) or invasive (e.g., pulmonary arterial or aortal sensors) and are restricted in their application and not suitable for everyday use. Therefore they are all limited to either experimental application or to specific conditions during intensive care therapy.

Beside the handling and the applicability of thermal probes it is of critical importance to estimate if the sites for measuring body *core* temperature in the past were the appropriate ones to assess body core temparture correctly. Whilst the brain (hypothalamus) is the "command central" of thermoregulation, the standard in physiological and clinical research on thermoregulation is still the recording with a semi-invasive rectal probe. Other ways to measure body core temperature more at the body's center are usually more invasive (see above) but more sensitive at the same time, while noninvasive means mostly remain closer to surfaces (e.g., skin), but their sensitivity drops. Therefore, measuring the rectal temperature seems likely to be a compromise even though it is not convenient and lacks applicability as well as sensitivity under extreme conditions in Space or in the cold, respectively.

In addition, temperature changes in the rectum are more variable due to gender differences. For example, in female the assessment of the circadian temperature rhythm in the rectum is less reliable due to the circatrigintan rhythm (28-day-rhythm) following the ovulation cycle affecting body core temperature profiles with a variance of 0.5 to 1.0°C according to the day of the cycle. This is a consequence of the anatomical proximity of the female gender apparatus to the rectum with an alternating uterine/vaginal blood circulation until the menopause which therefore can locally affect the temperature in the rectum in the range of 1°C at least. Another important impact for the reliable measurements of the the body core temperature results from the physiophysical effect of temperature regulation at different surrounding temperatures. This is explained by Aschoff saying that in hot (e.g., 35°C) environment the body shell is close to reflect the core temperature. In contrast, in more ambient or colder environments (e.g., 20°C), the body shell is colder as well and the external measurment of the core temperature is biased (Aschoff et al. 1971). But the head and hence the brain would not be included in that temperature changes because in that area the temperature will be stable, either under conditions of normal life at ambient temperatures as well as in extreme and cold environments. In the latter condition blood from the lower extremities will reduce also the temperature in the pelvis and the rectal temperature accordingly. In conclusion, the head temperature (and particulary the brain) seems to reflect best the "real" body core temperature as the center of temperature regulation (hypothalamus). The rear hypothalamus as a temperature regulating center receives its information about peripheral and central temperature receptors. Cold receptors in the skin and the spinal cord and warm receptors in the abdominal organs send signals about the lateral tractus spinothalamicus to the formatio reticularis and further to the rear hypothalamus (see Chap. 7). Warm sensors in the front hypothalamus measure,

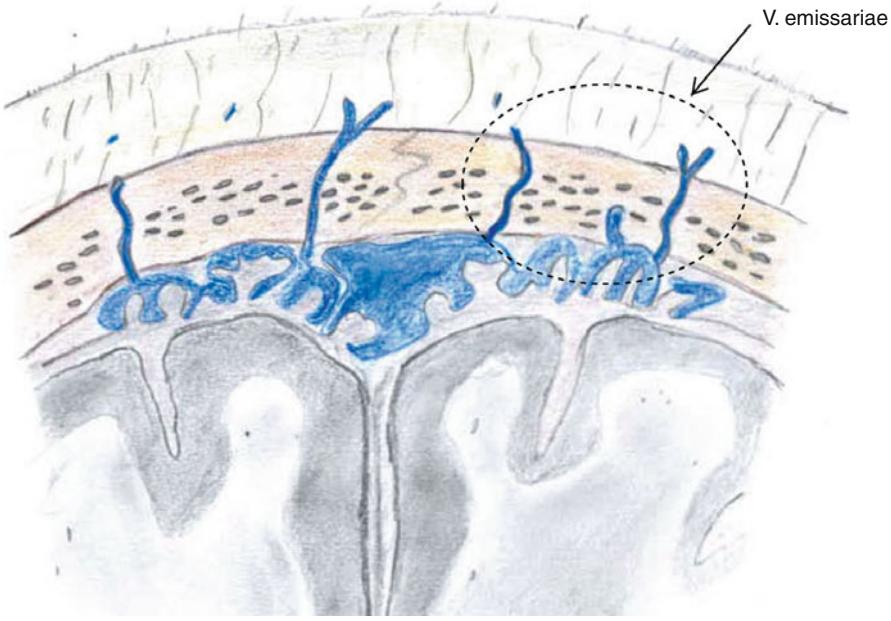

Fig. 23.1 Venae emissariae an evolutionary aspect in humans to regulate the body (brain) core temperature. Cross section of parts of the left and right brain hemispheres. Cerebral vein and V. emissariae (blue), skull (bright brown) and skin, artwork by M. Hörl, inspired by the Sobotta Atlas of Descriptive Human Anatomy, Urban & Fischer

in addition, the blood temperature (central receptors). In the hypothalamus the normal body core temperatur is "stored" (36.7°C). Divergences between that "set" temperature and peripheral changes will be regulated from that location (e.g., throughout in coldness increasing the metabolic rate) for the purpose of holding the brain and consequently the body core temperature stable. Consequently the ultimate anatomical site would be by far in the head, but which is of course impossible for ethical reasons. Therefore, the question is: "How to measure the brains', and hereby the body core temperature from outside the brain?" In this context one important anatomical connection between the brain and skin by the venae emissariae helps providing the "access" to the brain and core temperature (Fig. 23.1). This network of veins, which is only found in the cranium of humans, is involved in the temperature regulation of the brain. These vessels have originated and further developped as a function of the increasing brain volume and higher energy turnover together with the need to "remove" heat to maintain the brain temperature constant.

In brief, measuring body core temperature needs to fulfill the following prerequisites as the sensor: (1) Should not be disturbing for the subject, (2) should be able to access easily, (3) must comply to the basic hygiene standards, (4) should not be influenced by the environmental conditions, (5) should be sensitive enough to measure small changes in the subject, (6) its response time has to represent quantitative results, and (7) should assess temperature (changes) as quick as possible

Table 23.1 Exemplary types of sensors to measure body core temperature and their characteristics; Invasive temperature assessments in the aorta are not further explained in the text

Catheter aorta ascendens	"Gold Standard" – reference temperature, highly invasive
Esophageal	Invasive, good correlation to Gold Standard
Tympanic	Noninvasive, dependent on user and environmental influences
Temperature "Pill"	Semi-invasive, dependent on position within the body (gastrointestinal tract)
Rectal temperature	Invasive, good correlation to gold standard, slow reaction time, less compliance

(Shiraki et al. 1986; Hoyt and Friedl 2004; Smith 1980). These requirements will be discussed in the the following sections, beginning with the gold standard and coming to a new device which is the so-called: Double Sensor.

23.2 Current Status/Limitations

Currently there is no accurate and easy method to measure core temperature in a field setting and to a certain extent in the laboratory as well, particularly during long-term (>24 h) core temperature recordings in chronobiology (see Chap. 7).The definitions of various temperature measurements used in wearable body activity monitors are summarized in Table 23.1.

The relative advantages and disadvantages of core temperature measurement sites including the time response of the different kind of sensors, have been intensively discussed ever since the first benchmark investigations on this topic by Claude Bernard in 1876, and will be briefly described in the following section.

23.2.1 Sensors to Measure Body Core Temperature

The most common places for measuring core body temperature are the rectum, esophagus, and tympanum/auditory meatus and the gustatory.

23.2.1.1 Esophageal and Rectal Temperatures

Most thermal physiologists agree that the esophageal temperature is the best noninvasive index of core temperature for humans. It responds rapidly to changes in blood temperature elicited by extracorporeal circulation (Shiraki et al. 1986; Molnar and Read 1974) and body cooling by anesthesia (Cooper and Kenyon 1957). The esophageal temperature is obtained by inserting a catheter, containing a thermocouple or thermistor, through the nasal passage into the throat and then swallowing it. It is best used in research settings, but it is highly problematic in clinical or field assessments, this holds for the other core body temperatures as well (rectal and tympanic/auditory meatus), they are all impractical to use in the field (Steinman et al. 1987; Buller et al. 2005). The rectal temperature is obtained by inserting a temperature sensor a minimum of 5 cm past the anal sphincter, because temperature measure-

ments are uniform within the rectum from 5 to 27 cm past the anal sphincter (Nielsen and Nielsen 1962; Greenleaf and Castle 1972). During exercise it takes approximately 25–40 min to achieve a steady-state rectal temperature value (Aikas et al. 1962; Nadel and Horvath 1970; Greenleaf and Castle 1972). These steady-state rectal temperatures are usually ~0.4°C (Mairieaux et al. 1983) higher than the mean skin temperatures (Wenger 2001) and 0.2–3°C higher than simultaneously measured nasopharyngeal and esophageal temperatures (Cranston et al. 1957; Saltin and Hermansen 1966; Saltin et al. 1970; Nadel and Horvath 1970).

The esophageal and rectal temperatures–despite their limited reliability in specific conditions as described above – have been considered to be so far largely independent of the environmental temperature (Strydom et al. 1965; Stolwijk et al. 1968; Saltin et al. 1970; Gunga et al. 2005;). As a result, the steady-state rectal temperature provides a good index to assess body heat storage (Strydom et al. 1965; Saltin and Hermansen 1966). The main problem referred to rectal temperature measurements results from its slow response slope in comparison to the other measurement sites, an observation that has been proven again recently in 60 patients who underwent a postoperative rewarming (Melette 1950; Braeuer et al. 1977, 2000). The reason for the slow response is probably (1) a low rate of blood flow to the rectum compared to other measurement sites (Molnar and Read 1974; Aulick et al. 1981) and (2) the mass of organs located in the body cavity. This greater mass of tissue in the lower abdominal cavity requires a far greater amount of energy to cause a rapid temperature change.

23.2.1.2 Tympanic Temperature

Tympanic temperature is obtained by inserting a small temperature sensor into the ear canal and advancing it until it rests against the tympanic membrane. Proper placement is determined by the subject hearing a sound when the temperature sensor touches the tympanic membrane. Some subjects find this contact to be uncomfortable (Brengelmann 1987). In addition, there are reports of the temperature sensors perforating the tympanic membrane (Dickey et al. 1970; Wallace et al. 1974; Tabor et al. 1981). Because of the potential discomfort and trauma, as well as the placement problems associated with tympanic measurements, some investigators have chosen instead to measure the temperature of the external auditory meatus. For this measurement, a temperature sensor is placed in an ear plug and inserted into the external auditory meatus. Placement of the temperature sensor is important, since there is a substantial (~0.5°C) temperature gradient along the wall of the meatus. In addition, several studies have shown that tympanic/auditory meatus temperature measurements do not provide a reliable index of the level of core temperature during either rest or exercise (Nadel and Horvath 1970; Greenleaf and Castle 1972; Marcus 1973a, b; McCaffrey et al. 1975; U.S. Army 2003). Depending upon the environmental conditions, tympanic/auditory meatus temperature values can be lower or higher than simultaneously measured steady-state rectal (Greenleaf and Castle 1972) and esophageal temperature values (Marcus 1973a, b; McCaffrey et al. 1975). In addition, local head heating and air flow to the face will bias the temperature of the external meatus (Nadel and Horvath 1970; Greenleaf and Castle 1972; Marcus 1973a, b; McCaffrey et al. 1975; Sawka and Wenger 1986).

23.2.1.3 Temperature Pill

The best and most reliable method of assessing thermal state in operational environments is direct measurement of core body temperature by using the network-enabled ingestible core temperature sensor. However, the use of a core temperature ingestible sensor is impractical for routine use, so these devices are reserved for use during high thermal stress missions, in case of heat injuries such as in hyper- and hypothermia (Weller and Withey 2005). The disadvantage of such a device is the moving of the temperature pill in the gastrointestinal tract. If the pill locates in front of the liver – a highly metabolic active organ – the temperature will be higher than in the ileum or in the jejunum and therefore the continuity of temperature recordings will be not accurate. Therefore, it is assumed to get different temperatures from the "body core" but as in proximity to the liver higher body core temperature will be measured. On the other hand in the intestinal distal intestinal tract, e.g. in the colon, the body core temperature might be underestimated.

23.2.1.4 Skin Temperature

Although the skin surface is more easily accessed than the other body core temperature sites mentioned above, this measurement site includes some important confounding factors, because the skin temperature is largely affected by cutaneous blood flow and sweat evaporation. Furthermore, environmental changes such as air temperatures, humidity, wind speed, and radiation will alter the skin temperature. That is why thermal physiologists prefer to determine mean skin temperature, a sum of weighted individual skin temperatures taken at up to 16 different skin surface sites, or for certain questions, even more (Ramanathan 1964; Mitchell and Wyndham 1969; Murgatroyd and Hardy 1970; Fox and Solman 1971). For the field purpose, such a complex, heavily wired temperature measurement setup is highly impractical. Other concepts have shown that it is very difficult to use a single skin temperature sensor, even when it is combined with the body mass index (BMI), the knowledge of the clothes worn, to result in a reliable and accurate body core temperature (Yokota et al. 2005).

23.2.2 Heat Flux Sensor (Double Sensor)

23.2.2.1 Principles and Proof of Applications

Clearly, the rectal temperature and the radio pill are the most widely used and accepted by physiologists for core temperature recordings. In most cases the core temperatures are obtained by inserting a temperature sensor a minimum of 5 cm past the anal sphincter or by taking a radio pill with a transmitter system, respectively. Nevertheless the rectal and the radio pill temperature have some shortcomings by its own. Temperature recordings are uniform within the rectum from 5 to 27 cm past the anal sphincter (Abrams 1981; Benedict 1911; Karlberg 1949; Nielsen and Nielsen 1962). During exercise it takes approximately 12–40 min to achieve a steady-state rectal temperature value (Melette 1950; Aikas et al. 1962; Nielsen and Nielsen 1962; Saltin et al. 1970; Greenleaf and Castle 1972; Saltin 1972). These

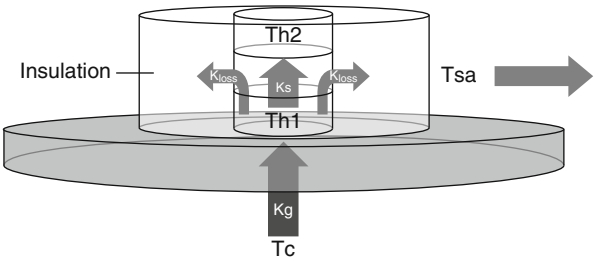

Fig. 23.2 Schematic side view of the Double Sensor. It contains two temperature sensors; an insulation disk placed in between which thermal (Gunga et al. 2008a) conduction coefficient (*Ks*) is known. Core temperature (*Tc*), thermal conduction coefficient of human tissue (*Kg*), skin temperature (*Th1*), second temperature sensor for measuring the heat flux (*Th2*), lateral heat loss (K_{loss}), temperature on the outside area of the insulation (*Tsa*); middle gray – human skin (Dr. Koch, Dr. Sattler, Fa. Draeger Werke, Lübeck, Germany)

steady-state rectal values are then generally ~0.2°C higher than simultaneously measured esophageal temperature values (Cranston et al. 1954; Nielsen and Nielsen 1962; Saltin and Hermansen 1966; Saltin et al. 1970) and they are usually quite independent of the environmental temperature changes (Stolwijk et al. 1968 Nadel and Horvath 1970; Greenleaf and Castle 1972). As a result, the steady-state rectal temperature provides a good index to assess body heat storage (Strydom et al. 1965; Saltin and Hermansen 1966). The main disadvantage with the rectal temperature is that it is very slow to respond to changes in blood (Melette 1950) and other core temperatures. The reason for the slow response of the rectal temperature to thermal transients is probably a low rate of blood flow to the rectum compared to other recording sites (Molnar and Read 1974; Aulick et al. 1981; Sawka et al. 1996). The slow response time makes rectal temperature a poor core temperature index for estimating the input to the thermoregulatory controller (Eichna 1949; Gerbrandy 1954; Saltin et al. 1970).

Gunga et al. (2005, 2008a, 2009a) have introduced a combined skin temperature and heat flux sensor (Double Sensor) (Patent No. DE 100 38 247, DE 101 39 705, 2003). In contrast to similar methodological attempts in the past, this zero heat flux sensor principle (Teunissen et al. 2011) has been miniaturized and used without extra heating, and has been specially sealed (Kimberger et al. 2009; Opatz 2010). The sensor draught works with two temperature pills which are on top of each other. Using the heat flux, which is an energy flow of heat through the temperature pills, the direction is from the skin away to the environment (Fig. 23.2), the body core temperature can be calculated by an algorithm: Tc = Th1 + Ks/Kg * (Th1 – Th2) (Gunga et al. 2008). The Double Sensor is placed at the vertex of the head or on the lateral forehead and has been tested under various physical and environmental conditions (i.e., changing workloads and ambient temperatures of 10°C, 25°C, and 40°C). A measurement comparison of the new sensor with the rectal temperature revealed that the Double Sensor (1) differed between −0.16°C and 0.1°C from the average of the rectal temperature, (2) showed, with increasing ambient temperatures, increasing con-

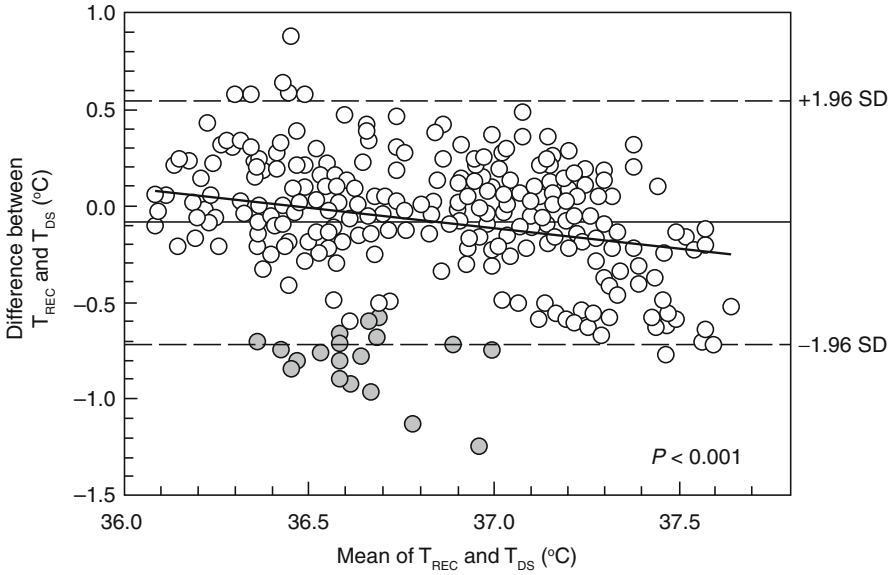

Fig. 23.3 Diagram of the difference between the methods against the averages of the methods (T_{REC} rectal temperature; T_{DS} Double Sensor). Statistical method according to Bland-Altman shows the evaluation of the Double Sensor underestimated compared with rectal temperature measurement at lower temperatures and overestimated at higher temperatures

cordance correlation coefficients (CCC) (all work periods: 10°C = 0.49; 25°C = 0.69; 40°C = 0.75), and (3) exhibited a more rapid response to the body core temperature changes for all resting periods at all ambient conditions, as compared to rectal temperature ($P < 0.01$) (Gunga 2008) (Fig. 23.3). The Double Sensor can possibly be integrated in the helmet (Werner et al. 2009, 2010) or also into protective clothes and can measure the body core temperature accordingly. In the last years, the applicability and reliability of the Double Sensor could be presented under different physical and environmental conditions. If these data from the heat flux sensor are combined with cardiovascular data (e.g., heart rate), then it might be possible to predict thermal physiological strain (PSI) in humans, according to Moran et al. (1998). However, they observed limitations of the heat flux sensor in cold environments that need to be addressed and investigated further. The main components of the Double Sensor unit hardware and the position of the Double Sensor are shown in Fig. 23.4.

In a bed rest study (Berliner bed rest study – BBR2) the Double Sensor was compared against the rectal temperature admission to examine the circadian rhythm profile (Gunga et al. 2009a). The temperature data with the Double Sensor were highly comparable with the rectal temperature measurement. The dispersion of the measured values is shown in Fig. 23.5. In summary, with regard to the great demand for developing an easy-to-operate and noninvasive technology to measure core body temperature in humans, we have evaluated the new skin temperature and heat flux measurement device to monitor body core temperature changes during 24 hours at – 6°– head-down tilt bed rest. In this regard, it seems noteworthy

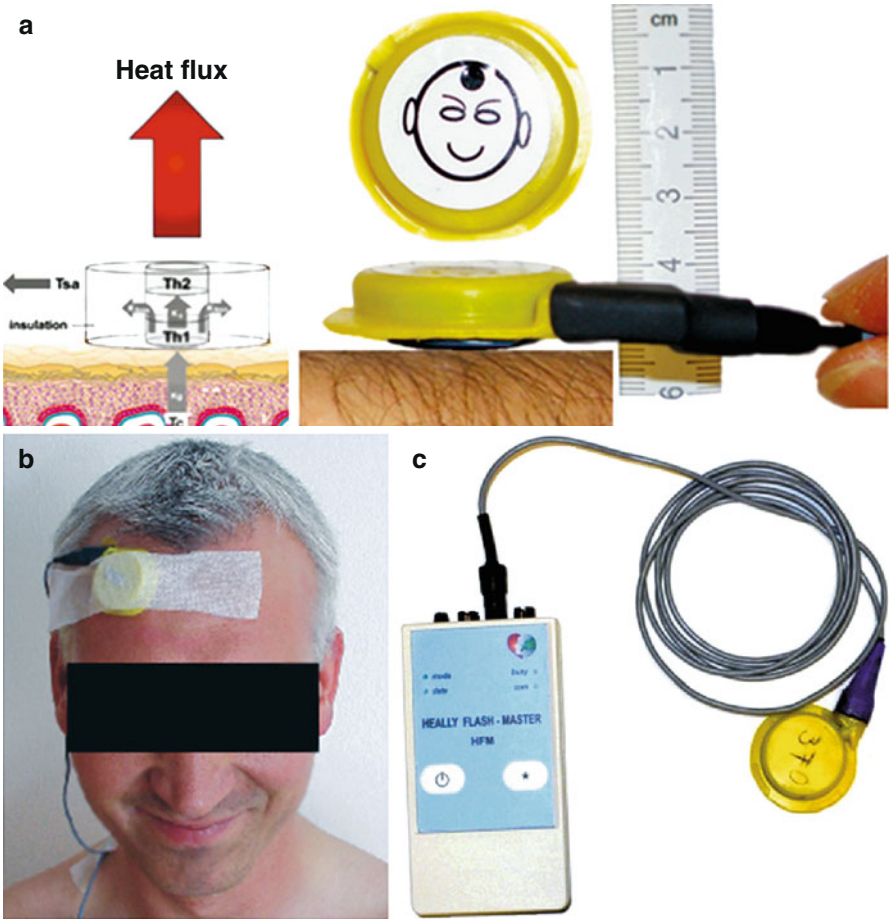

Fig. 23.4 The Double Sensor for the measurement of body core temperature could be stuck by a tape on the forehead and is connected by a cable with the HealthLAb®-System (Fa. Koralewski, Hambühren, Germany, Fa. SpaceBit, Eberswalde, Germany)

that rectal temperature recordings themselves are not without error. Furthermore, it was found that circadian body core temperature profiles could be well approximated by the Double Sensor. Thus, even if the available pool of subjects is small, we are confident that the Double Sensor technology is accurate enough for detecting relatively small meaningful differences in circadian rhythm profiles compared to rectal temperature recordings. In addition to this, further approaches have been undertaken taken to evaluate the Double Sensor device in deep hypothermia (Werner et al. 2009, 2010), where promising results could be obtained. In conclusion, the knowledge and technological development gained from the study could promote applications of continuous core body temperature measurements in clinical, occupational, sports, and environmental medicine on earth and in space (Werner and Gunga 2008). The Double Sensor device might be an effec-

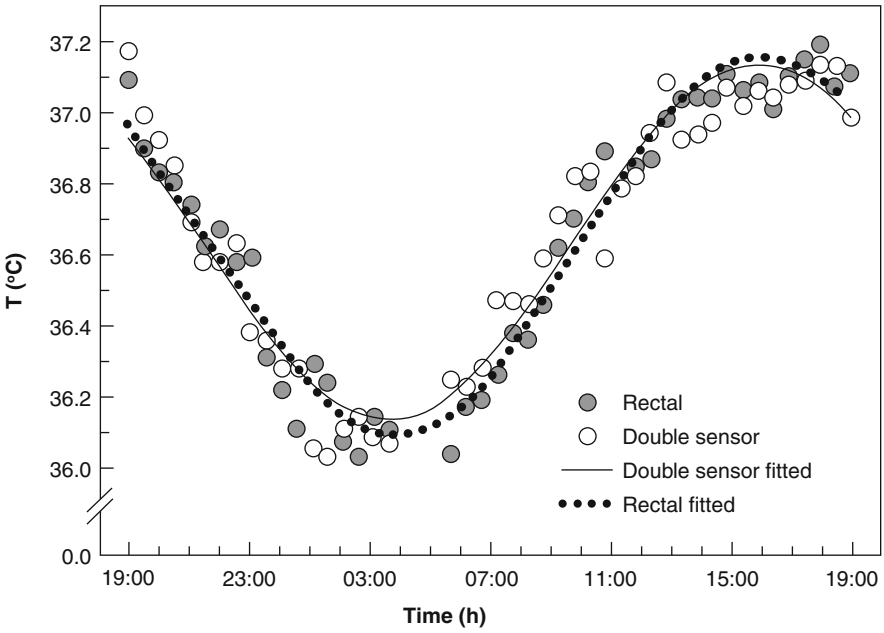

Fig. 23.5 Scatter plot of rectal temperature and body core temperature measured by Double Sensor under rest conditions (Berlin Bed Rest Study – BBR 2)

tive, noninvasive and easy-to-operate technology to gain fundamental insights into cardiocirculatory regulation, thermoregulation, and circadian rhythms in humans (Werner 2011a).

23.3 Thermoregulation in an Extreme Environment – Space

23.3.1 ThermoLab – Thermoregulation Under Exercise Conditions

Humans have an endothermic metabolism and autonomous regulation mechanisms to ensure that the body core temperature for the vital organs in the body core (brain, heart, liver and kidneys) is approximately 37°C. In particular, different surrounding temperatures can challenge the temperature homeostasis and can alter the relationship of body core to body skin.

The thermoregulatory center functions act in the same way as a technical regulatory system; it compares the information coming from the thermo receptors with a preset target value, and, if there are variations, it activates the effector system, for example, supply of blood to the skin (see below), to regain the intrinsic target values preset by the hypothalamus. An even heat balance in humans is only ensured when the two decisive factors in the heat balance, heat production and heat loss, are balanced with each other. This is what is known as the so-called thermal neutral zone (Hardy and DuBois 1938; Kirsch 1979; Jessen 2000; Gunga 2004). Only when the appropriate balance between

heat production and heat loss is ensured, the human can feel comfortable with respect to his thermal homeostasis. This comfortable condition is achieved for a lightly clothed male adult by a temperature of 27°C (indifference temperature), given under atmospheric conditions at sea level with 60% relative humidity and slight wind movement. In contradiction, water, with its high heat transmission capability and high heat capacity, has an indifference temperature of 33.5–34.5°C when compared to air. This means that the autonomous and morphological adaptation permits humans only a very narrow range of temperatures in which it is possible to remain unprotected.

All the energy converted in the organism, unless it is needed for building new body substance or for physical work, is converted into heat. The following four mechanisms are available for the heat exchange between body and environment: (1) conduction (heat transfer in a resting body), (2) convection (heat transfer via moving medium such as air), (3) evaporation (evaporative cooling), and (4) radiation (long-wave infrared part of radiation). If it is necessary to increase heat loss, the peripheral blood circulation in the skin is increased (vasodilatation); if it is necessary to reduce heat loss, the blood circulation in the skin is constricted (vasoconstriction). Vasodilatation and vasoconstriction is also described as "dry" heat loss, as opposed to the "wet," the evaporative heat loss (evaporative cooling). If heat loss is too high, the body temperature can be raised by increased metabolic heat production (e.g., shivering).

Thus, convective heat transfer makes up to 1/3 of the total heat loss mechanisms at environmental temperatures of 30°C. Convective heat transfer from the skin to the environment also affects, in particular, the buildup of the boundary layer between skin and the surrounding air. In this 4–8 mm thick boundary layer, there is normally a laminar air flow, which in a standing human runs from the feet along the body axis to the head. Approximately 600 l of air circulate in this way per minute along the body; this upward streaming sheath of warmed air is called natural convection. At any skin area, the local heat loss depends on the temperature gradient, and the thickness and velocity of the sheath. The structure and thickness of this boundary layer make a decisive contribution to the thermal well-being of the human (micro-climate).

A lack of gravity impairs in a sustained (Yu and Yang 2000a and b) manner the natural share of convective heat transfer from the body surface as gravity, as the driving force for this convective heat transfer ceases to apply at the body surface along the body axis under microgravity conditions (Blanc et al. 2000; Kuhlmann 2000; Yu et al. 2000; Zhang et al. 2000). The changes in convective heat transfer for a candle and for the human body to the environment are shown in Figs. 23.6 and 23.7. These physical findings will affect the thermal comfort of the astronaut/cosmonaut under these specific conditions (Novak 1991; Qui et al. 1997, 2002), especially during extravehicular activities (EVA) (Clement 2003). Air temperatures in the Space Shuttle and the International Space Station ISS have been regulated between 18 and 29°C with a maximum humidity of 14 Torr and normobar-normoxic conditions (Messerschmid and Bertrand 1999; Seibert 2001). It is known from the experience of the cosmonauts on the space station MIR that the cosmonauts complain of thermal discomfort at the extremities (Polyakov et al. 2001, Polyakov, personal communication; Clement 2003). This can result from increased catabolism in the muscular mass in this area (muscular metabolism), a vasoconstriction/vasodilatation, change in fluid shifts, energy balance and/or a change in heat transfer (Stein 2000).

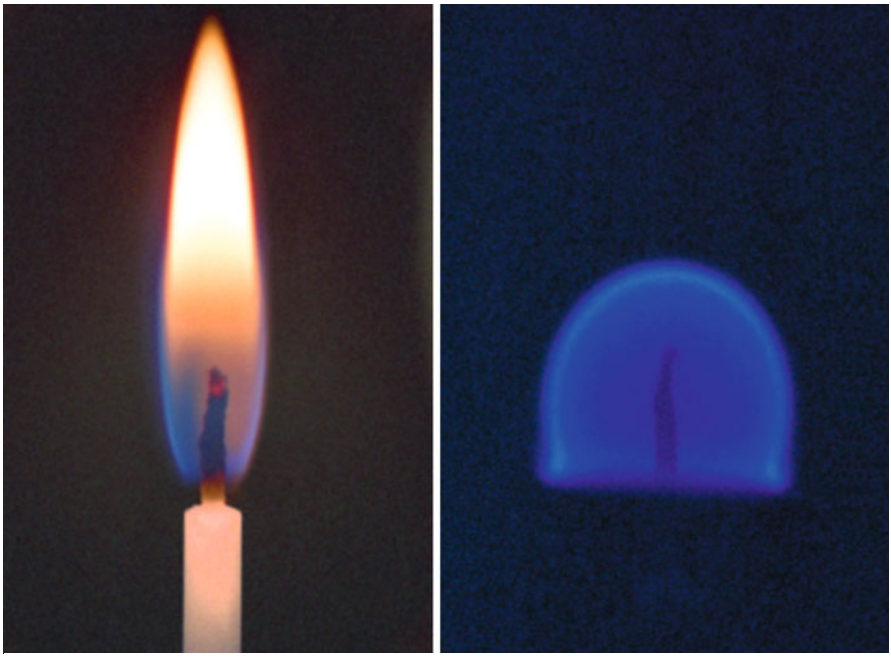

Fig. 23.6 A candle flame in Earth's gravity (*left*) and microgravity (*right*) showing that difference in the processes of combustion in microgravity. Image courtesy of NASA, Johnson Space Center (see http://www.nasa.gov/mission_pages/station/research/experiments/SAME.html)

Under these conditions the body core temperature can change rapidly, eventually reaching deleterious levels. Core temperatures recordings are quite difficult to achieve by inserting a thermosensor into the body until now. None of the methods are really applicable during daily terrestrial routines either in space. However, a thermosensor with its distinct advantageous, as described above, can be suitable for the following applications to assess stress effects in space: firstly to assess and to monitor the heat homeostasis under stressful environmental conditions in space and secondly, to achieve reliable assessments of changes of the circadian rhythm during spaceflight. The current study, "Thermolab," has been implemented now on the International Space Station (ISS), on February 2009, in combination with another experiment of the NASA (project DLR 50WB0724). It will lead to a better basic understanding of heat transfer and thermal regulation in humans under microgravity conditions. This new finding will help to support not only astronauts, but also, for example, Special Forces and fire fighters who share the risk of thermal heat strain, because they are frequently exposed to hot or cold environments especially during extravehicular activities (EVA), have to perform high physical workloads and have to wear heavy armor protective clothing against heat, cold stress, or radiation as well. Those findings might be also important for further long-time space explorations and activities such as EVA or missions to Moon and Mars.

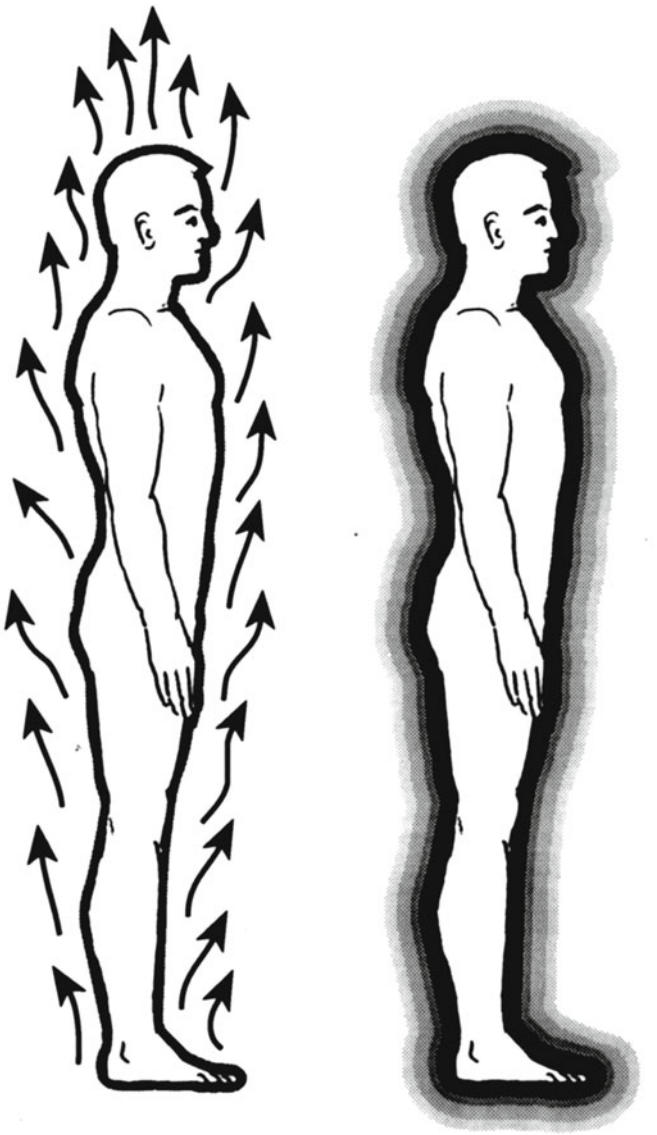

Fig. 23.7 The differences of being on Earth and in Space. The *left figure* shows the air flow around the body under normal conditions (600 l/min of air flow); the *right figure* shows the condition under micro-g in Space, the conductive heat transfer under these conditions is altered because of nonexistent air flow in Space

23.3.2 ThermoLab – Thermoregulation and Circadian Rhythmicity

Based on the previous and actual (MARS500, Werner 2011c) experience it was approved to use the Double Sensor during long-term spaceflights on ISS to establish temperature recordings in humans. This technology would be particularly useful for exploring changes in circadian rhythm during long-term mirco-g exposure. Double Sensor temperature recordings will obtained for a period of 36 h in 6 or 7 in-flight session during a 6 month stay on International Space Station (ISS). The measurements

could not be started in the evening as on earth because there is a light–dark rhythm for about 90 min, which means every 90 min you have a period of light and darkness. Therefore, it is expected that the circadian timing system (CTS), which is normally 24 h, will be affected by these rapid light–dark circuit. This may have an influence in the coordinated daily variation of physiological and psychological behaviors. A synchronized circadian rhythm is very important to health, emotion, and cognitive function (Monk et al. 1998; Manzey 2001). In this context, also human performance and symptoms of fatigue and concentration could be evaluated in further studies. Maybe one of the findings will be to bring adequate workplace illumination on ISS to adhere an as far as possible normal circadian temperature rhythmicity under micro-g conditions. This will have also an impact on the planning of Moon and Mars missions in the future.

23.4 Summary

Humans have an endothermic metabolism which is regulated autonomously with a body core temperature around 36.7°C ± 0.5°C. But there is absolutely a need for measuring body core temperature not only in patients but also in workers under extreme conditions during work. The measurement of body core temperature is not very easy. The more noninvasive you get the more insufficient the sensors will be. Furthermore, the discussion where the body core is located is an open issue. According to the center of thermoregulation, which is in the brain (Hypothalamus), temperature should be measured around there. But the invasiveness and therefore ethical reasons forbid bringing a sensor into the brain. Therefore the clear requirements for a sensor are to measure body core (brain) temperature noninvasively but very reliably, with a hardware that it easy to use and which can be worn comfortably. To fullfil theses scopes, a new heat-flux sensor was developped, the so-called double sensor. In a series of studies this sensor provided accurat and reliable body core temperature profiles by positioning the sensor on the forehead or vertex. Up to now the sensor has a very good correlation in hot environments, in coldness there were some open questions. The sensor could be worn under the clothes and during a whole working day. Because of this fact the sensor was launched to the International Space Station (ISS) to measure body core temperature before, during, and after long-term spaceflights (Werner 2011b). First results show the reliability and very interesting insights into the thermoregulation under conditions of weightlessness.

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Flow Cytometry Methods to Monitor Immune Dysregulation Associated with Spaceflight

24

Brian Crucian and Clarence Sams

24.1 Technical Background

Flow cytometry is a laser-based technique for scanning and evaluation of suspended cells or other particles. A Nobel Prize was never awarded for this breakthrough; however, it has evolved into an extremely important technology in both research and clinical medicine. Flow cytometers have an extremely rapid throughput, and can analyze thousands of individual cells per minute. Typically, they have a laser excitation source (most commonly 488 nm), and multiple photomultiplier tube detectors (PMT) for emitted light. Cell samples may be stained with various fluorescent antibodies to other dyes to identify cell surface proteins, intracellular markers, cell function, or other cellular targets. Upon cell excitation, the light emitted by the various fluorochromes is directed to the appropriate PMT via a network of long- and short-pass filters, dichroic mirrors, and band-pass filters. The number of colors visible to the cytometer (and thus the number of parameters that can be measured) is defined by the number of PMTs. In addition, excitation laser light that has been "scattered" by the target cell, regardless of any fluorochromes present, may also be measured. Laser light scattered in near opposition to the excitation source is defined as "forward scatter," and generally corresponds to cell size. Light scattered at approximately 90° to the excitation source is defined as "side scatter", and is a measure of cellular granularity. Together, the scattered light parameters may be visualized in bivariate dot plots. This resolves the basic peripheral blood leukocyte populations, granulocytes lymphocytes, and monocytes by scatter properties alone into distinctly visible populations prior to fluorescence measurements. This resolution allows identification, or "gating," of distinct leukocyte populations by scatter properties, to analyze their fluorescence emission properties separately. Thus, a four

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color cytometer with forward and side scatter, essentially becomes a 6-parameter instrument, and allows measurements of all resolvable cell populations to occur simultaneously. Cell populations may also be gated off fluorescence expression, for populations with overlapping scatter properties. For example, T cells may be resolved from within the lymphocyte population (also containing B and NK cells) by expression of CD3. Gating by immunoscatter does reduce the remaining fluorescence channels available for data acquisition.

Samples for flow cytometry analysis must be prepared prior to running the samples on the instrument. For blood analysis, this usually includes the basic steps of WBC staining, RBC lysis, and sample fixation/stabilization. The quality of the sample preparation relates directly to the quality of data generated by the cytometer. The basic blood staining described above may be employed to resolve and determine relative percentages of all major peripheral blood leukocyte subsets ("phenotyping"). However, since classical flow cytometers suspend particles in sheath fluid, they do not determine absolute counts. This is one distinction from hematology instruments, which will derive absolute counts as part of a complete blood count (CBC), but will not stain cells or measure emitted light. Hematology instruments can also resolve bulk populations based on size/scatter alone. Some newer miniaturized cytometers, including instruments from Guava and Partec, do not suspend cells in sheath fluid and thus can be used to determine absolute counts (Crucian 2005). By employing variation in sample preparation, including cell culture followed by analysis of the cultured cells, various measures of immune cell function may be measured by flow cytometry. Examples include the expression of cellular activation markers, determination of cytokine-secreting cells (permeabilization, intracellular cytometry), and measurement of secreted cytokine production. Measures of secreted analytes do not analyze cells, but use fluorescent bead technology as a capture platform, followed by staining with a fluorescent detection antibody. Various bead populations are "gated," and concentration of the secreted analyte may be determined.

Immune system dysregulation is associated with spaceflight, and a review of the phenomenon may be found elsewhere in this volume (see also Chaps. 9–14). A variety of flow cytometry techniques described above have been used to assess immune parameters during, or immediately following, spaceflight. Variations between the designs of these studies include simple postflight sampling of human or animal subjects, subject sampling during orbit for postflight cytometry analysis, and cell culture during orbit followed by postflight cytometry analysis. During one pilot study conducted during the MIR-18/STS-71 mission, staining, lysing, and preservation of Astronaut whole blood samples was performed in-flight, and the stained cell samples were returned for postflight analysis (Sams et al. 1999). The relative ease and extensive versatility of flow cytometry makes it an ideal instrumentation platform for definition of spaceflight-associated immune dysregulation. This chapter discusses flow cytometry assays that have been applied to, and identify immune changes associated with, spaceflight.

24.2 Flow Cytometry Assays Used to Define Immune Changes Associated with Spaceflight in Humans or Animals

24.2.1 Peripheral Leukocyte Distribution

Measurements of the distribution of the peripheral blood leukocyte subsets (or splenocyte/marrow subsets, from mice or rats) is one of the most common flow cytometry techniques and has been used in many spaceflight studies. Alterations in the distribution are frequently reported, and reflect in vivo immune changes that may have clinical significance, similar to that of the complete blood count (CBC) measurement. Increases in immune cell populations may reflect immune activation or clonal expansion and proliferation. Decreases may reflect cell migration out of the blood to the periphery or a localized site of inflammation, or blockades in cell maturation processes. As the number of available fluorochromes and channel capabilities of flow cytometers has increased over the last two decades, the resolution of immune cell subsets has also increased. Earlier in the space program, simple "bulk" subsets were assessed on 2–3 color instruments. These may have included granulcoytes, lymphocytes, monocytes, lymphocyte subsets, and the CD4:CD8 ratio. Today, 4–6 color instruments with distinct fluorochromes make possible the analysis of various activated populations, and "fine" T cell subsets, such as cytotoxic effector CD8+ and central/effector memory T cells. For example, T cells may be measured by simultaneously assessing CD45 and CD3, and memory T cells: naïve T cell subsets may be assessed by CD45RA/RO expression, whereas central memory T cells require CD3, CD8, CD45RA, and CD62L or CD11a. Studies by Roederer and others have indicated that diseases are often accompanied by changes in the number or function of "fine" lymphocyte subsets, even if changes in the bulk lymphocyte populations are not evident (De Rosa et al. 2001). Other "fine" leukocyte subsets are being identified, some with potential clinical significance, as the field of clinical immunology ever progresses. Some representative spaceflight studies employing phenotype analysis are discussed below.

In an example of an early assessment of bulk leukocyte subsets, Stowe et al. performed a postflight WBC and differential on short-duration Space Shuttle astronauts (Stowe et al. 1999). A postflight elevation in WBC and granulocyte percentage was observed. This is an almost universally reported phenomenon, widely believed to represent demargination associated with reentry and landing stress. A subsequent study from our laboratory assessed lymphocyte subsets, the T cell CD4:CD8 ratio, and basic memory naïve T cells as defined by CD45RA+/– following short-duration spaceflight (Crucian et al. 2000a). The data indicated a decreased percentage of T cells, an increased CD4:CD8 ratio, and variable changes in memory T cell levels.

Two subsequent studies used flow cytometry to assess leukocyte distribution and stress-related parameters in Space Shuttle astronauts on missions with varying durations to determine the effects of mission duration. Mills et al. reported that the

WBC, granulocytes, monocytes, B cells, and CD4+ T cells were elevated, and NK cells were decreased postflight. The alterations were more pronounced following 1 week missions, as opposed to 2 week missions, and the authors concluded that shorter missions were associated with predominantly sympathetic responses, potentially attenuated following longer missions (Mills et al. 2001). Stowe et al. followed this study by assessing neuroimmune responses in astronauts participating in 9 or 16 day missions. The results confirmed that sympathetic nervous system responses dominate following short space missions, with longer missions characterized by glucocorticoid-mediated changes (Stowe et al. 2003).

During the STS-108 Space Shuttle mission in 2001, Peacut and Gridley extended the use of flow cytometry to analyze minute cell suspensions and blood from spaceflown BALB/c mice, analyzing peripheral blood, marrow, and splenic leukocyte subset changes. Pecaut et al. indicated that no differences were observed in the general circulating lymphocyte proportions. However, changes in spleen leukocyte subsets were reported, including increased lymphocytes, decreased granulocytes, increased B cells, and decreased CD4+ T cells. In the same animals, there were proportional increases in marrow T cells and decreases in B cells (Pecaut et al. 2003). In the same animals, Gridley et al. reported percentages of CD25+ lymphocytes, CD25+ T cells, and NK1.1+/CD25+ NK cells were elevated in the STS-108 mouse subjects, whereas CD71 expression was decreased (Gridley et al. 2003). In these studies, the traditional bulk leukocyte distribution was expanded to include activation markers and adhesion markers, as well as organ-specific assessments of leukocyte distribution.

Subsequent to the 2001 studies, the authors performed a follow-up study during the STS-118 Space Shuttle mission in 2007. Gridley et al. reported that levels of T cells and B cells were reduced, whereas NK cell levels were elevated in the spleen (Gridley et al. 2009). Ortega et al. reported on a comprehensive marrow assessment, where flow cytometry was used to assess expression of molecules that define the maturation/activation state of cells in the granulocytic lineage. The molecules used were Ly6C, CD11b, CD31, Ly6G, F4/80, CD44, and c-Fos. There were no composite phenotypic differences between the total bone marrow cells isolated from spaceflight and ground control mice, however there were subpopulation differences in several markers. In particular, an elevation of CD11b suggested neutrophil activation in response to landing, and decreases in Ly6C, c-Fos, CD44, and Ly6G with an increase in F4/80 suggested marrow cells from the spaceflight mice were more differentiated (Ortega et al. 2009).

In a 2008 post-flight assessment of both short duration and long duration (6 month, International Space Station) astronaut crew members, our laboratory expanded the traditional “bulk” subset analysis to include “fine” T cell subsets. These included central memory/effector memory CD8+ T cells subsets, cytotoxic/senescent CD8+ T cell subsets, and various constitutively activated T cell subsets. These studies were conducted using 5-color cytometry. In addition to the commonly observed changes bulk subset alterations, crew members displayed elevated levels of both CD4 and CD8 memory T cells (defined as CD45RO+), reduced levels of early senescent (CD57+) and late senescent (CD28-) CD8+ T cells, and reduced levels of terminally differentiated CD8+ T cells (Crucian et al. 2008, Chap. 12).

Because postflight measurements may be altered by stressful effects of landing and readaptation, phenotype, T cell activation and virus-specific immunity are currently being analyzed via flow cytometry in a subsequent in-flight study onboard the International Space Station. For this study, whole blood samples are being collected near to undock and return of a visiting US or Russian spacecraft, followed by terrestrial cell culture and flow cytometry analysis (Crucian et al. 2010).

In general, the studies described above demonstrate the utility of assessing leukocyte and lymphocyte subsets, as well as adhesion markers, activation markers, etc. for monitoring spaceflight associated immune dysregulation in humans and rodents. The resolution of subsets being monitored has advanced as cytometer technology and fluorochrome/dye development has advanced. It should be noted that changes in constitutive subset levels reflect in vivo immune changes, and thus may represent more individualized responses associated with adverse clinical reactions, than with generalized immune functional changes.

24.2.2 General Immune Cell Function

As several recent studies have demonstrated, measurements of immune function are proving very important in defining what immune dysfunction exists during spaceflight. Leukocyte subset measurements assess in vivo distribution shifts, and by default in vivo immune responses. The variables that influence human immunity in space, microgravity, physiological stress, isolation, altered nutrition, etc., are not necessarily related to pathological processes. Hence, they are much more likely to influence cellular function as opposed to cellular distribution. It is possible that crew member leukocyte distribution may be completely unaltered during flight, yet if immune cell function is (persistently) compromised, a clinical risk may still exist. Several investigators have reported altered granulocyte, monocyte, NK, and T cell function associated with spaceflight, and some of the current techniques for measuring cell function (and used in the cited spaceflight studies) employ flow cytometry. Some representative citations follow. Note that intracellular cytokine production, also a functional measurement, will be covered in the following section.

Kaur et al. performed a postflight assessment of monocyte oxidative burst capacity, a measure of monocyte function. Oxidative burst was determined by flow cytometry using the Fc OxyBURST Green assay reagent, which consists of bovine serum albumin covalently linked to dichlorodihydrofluorescein and complexed with purified rabbit polyclonal anti-BSA IgG antibodies to yield a multivalent particulate immune complex. When this immune complex binds to the Fc receptors of monocytes, the nonfluorescent H2DCF molecules are internalized at 37° but not at 4° within the phagovacuole of the activated monocytes, and subsequently oxidized to green fluorescent dichlorofluorescein. The data indicated that following 5–11 days of spaceflight, astronauts' monocytes exhibited reduced capacity to elicit an oxidative burst (Kaur et al. 2005, see also Chaps. 8–14).

T cell activation initiates when the T cell receptor (TCR) is triggered in an antigen-specific fashion in the context of the major histocompatibility complex

(MHC), with additional triggering of appropriate co-stimulatory molecules. During the process of full activation to clonal expansion, a >72 h series of events takes place. This process begins with nearly immediate transduction of intracellular signals to the T cell nucleus, involves changes in membrane potential and intracellular pH, and changes parameters such as receptor distribution and mobility, cytoskeletal rearrangement, alterations in the molecular conformation of adhesion molecules, and results in activation of adenylate cyclase, ATPase, and other membrane-associated enzymes. Within the first few hours there is coalescence of patched receptors into a cap and expression of preformed CD69 molecules on the T cell surface. Within 24 h secretion of IL-2 begins, there is upregulation of the IL-2 receptor (CD25) on the cell surface and IL-2 mediated autocrine activation initiates. By 36–72 h blast transformation begins, accompanied by expression of HLA-DR on the cell surface. Note that several of these changes may be detected by flow cytometry, especially tracking the progression of T cell activation via the expression of CD69, CD25, and HLA-DR. In fact, such monitoring may essentially replace the previous gold standard of 3H determination of T cell proliferation. In a prior study, our laboratory monitored early T cell activation potential in US astronaut crew members, both short and long duration, immediately following spaceflight. The data showed that long-duration crew members had significantly blunted T cell function postflight, whereas T cell function was actually increased postflight for short-duration crew members (Fig. 24.1, Crucian et al. 2008). The difference was attributed to the immunostimulatory effects of acute stress in less deconditioned crew members, versus the effect of chronic stress in severely deconditioned crew members. Since cultured cells may be analyzed by flow cytometry as easily as whole blood, there is no real limit to the functional measures that may be performed by flow cytometry.

In addition to postflight assessments of crew members, or postflight processing of in-flight samples, spaceflight effects may also be studied by culturing cells on-orbit. Following culture, astronauts may preserve the cultured cells by a variety of means, to be followed by ground-based cytometry analysis upon return to earth. Hashemi et al. investigated T cell progression during the first 24 h of activation by culturing T cells during spaceflight onboard the Biorack facility, Space Shuttle missions STS-81 and 84. T cells were stimulated with soluble antibodies to CD3 to trigger the TCR. Similar to the postflight astronaut study described above, expression of both CD69 and CD25 were measured by flow cytometry. Culture during spaceflight showed a dramatic reduction in expression of both CD69 and CD25 following activation (Hashemi et al. 1999). Complementation of TCR-mediated signaling by phorbol ester restored the ability of the T cells to become fully activated, indicating a protein kinase C (PKC)-associated pathway could be compromised during microgravity conditions.

In an example of the high versatility of flow cytometers, our laboratory has developed an improved flow cytometry assay for NK cell function that is being used during a current ISS in-flight study. The previous standard assay for NK cell function involved co-culturing "target cells" (K562 cell line) and human subject peripheral mononuclear cells (containing NK cells), to induce NK cell killing of the target cells. The target cells were radio-labeled, and killing was determined by measuring radioactivity released by the lysed target cells into the culture medium. Caveats to this assay include a varying number of NK cells within the peripheral blood mononuclear cell (PBMC) population, and

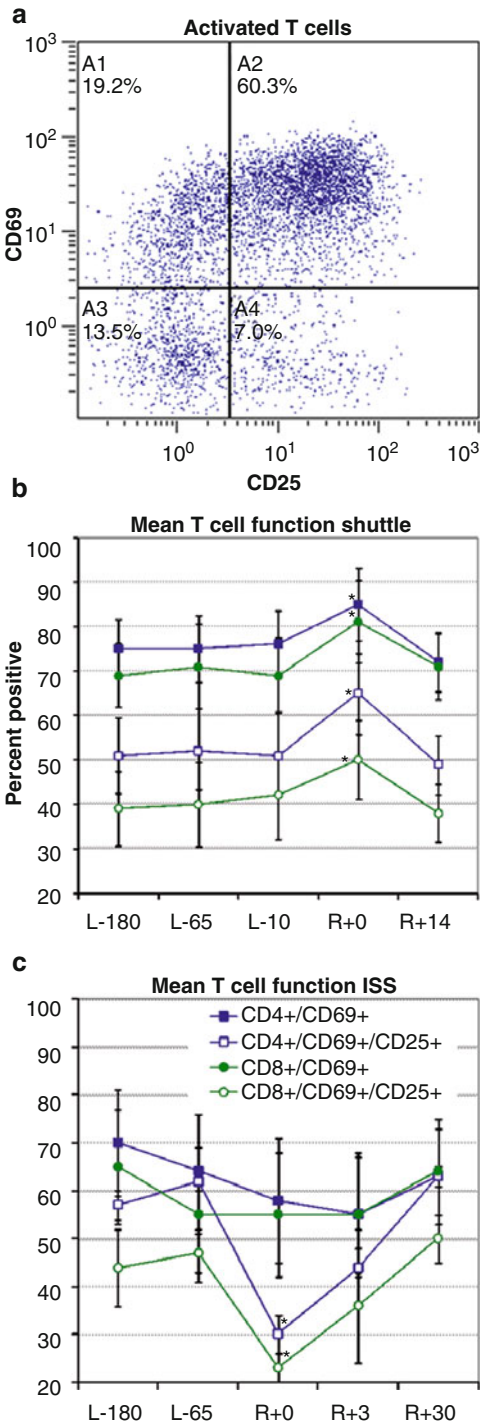
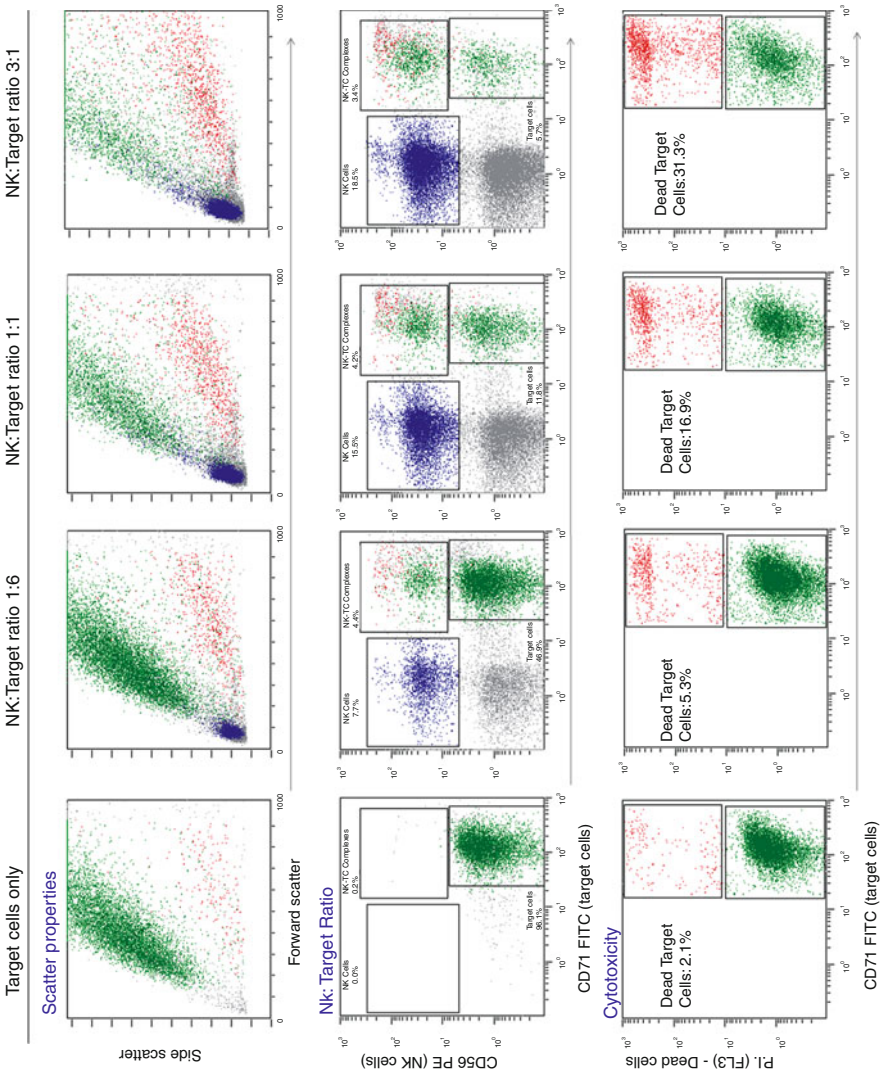


Fig. 24.1 T cell function by flow cytometry in Shuttle and ISS astronauts. Early blastogenesis of T cells, characterized by the expression of cell surface CD69 and CD25, may be measured via flow cytometry. This is a measure of the early events associated with T cell activation and may be considered a determination of cell function. (**a**) Representative dot plot following CD3/CD25 stimulation of T cells; representative postflight Shuttle (**b**) and ISS data (**c**). (Crucian et al. 2008)


Target cells only
NK:Target ratio 1:6
NK:Target ratio 1:1
NK:Target ratio 3:1
Scatter properties
Side scatter
Forward scatter
Nk: Target Ratio
CD56 PE (NK cells)
CD71 FITC (target cells)
Cytotoxicity
PI. (FL3) - Dead cells
CD71 FITC (target cells)
Dead Target Cells:2.1%
Dead Target Cells:5.3%
Dead Target Cells:16.9%
Dead Target Cells:31.3%

the requirement for large numbers of subject cells to co-culture varying ratios of PBMC: target cells in order to generate a kinetics curve for NK activity. For our current spaceflight study, the numbers of available astronaut cells are very low. For this reason, and to eliminate the requirement to work with radioactive materials, a nonradioactive flow cytometry method has utility for spaceflight studies. Nehlsen-Cannarella et al. developed an early nonradioactive flow cytometry assay, which used membrane dye labeling to identify target cells (Chang et al. 1993). Target cells were still incubated with subject cells; however, killing was measured by propidium iodide (PI) intercalation into target cell DNA via red fluorescence. To meet the requirements of our spaceflight study, we enhanced the assay by labeling NK cells with phycoerytherin (PE) labeled anti-CD16/56 antibodies, labeling the target cells with fluorescein isothiocyanate (FITC) labeled anti-CD71 antibodies. Killing is still determined by membrane permeation of PI via red fluorescence. Thus, a three color cytometry assay is created which can positively identify exact numbers of NK cells (not bulk PBMCs), live target cells and dead target cells (Crucian et al. 2006b ISAC, Fig. 24.2). This improved assay is appropriate for spaceflight studies, as minimal crewmember cells may be used. In addition, although measuring killing across multiple NK: target ratios is beneficial, it is not absolutely required since the precise number of astronaut NK cells and target cells is known. Using this method, an accurate killing percentage normalized to any NK cell value may be derived without assuming the PBMC population to accurately represent the NK population.

24.2.3 Cytokine Assays

A separate outcome from immune cell activation, distinct from the blastogenesis-related changes described above, is cytokine production. Cytokines are small molecules secreted by immune cells which cause effects in other immune cells. Essentially they are the hormones, or communication messengers, of the immune system. Cytokine biology is incredibly complex, with dozens of cytokines secreted by a very diverse network of specific immune cell subsets. Examples of cytokine categories include the pro-inflammatory cytokines of the innate immune system, and the Th1, Th2, Th17 cytokines secreted by various T cell subsets. Following cell activation during mitogenic stimulation culture, techniques such as ELISA or cytometric bead

Fig. 24.2 A nonradioactive method for determining NK cell function by flow cytometry. Separate data are presented for four cell cultures containing free target cells and 3x increasing NK:target ratios (columns left to right, respectively). Fluorescent antibody staining is used to positively identify target cells (CD71 FITC/*green*) and NK cells (CD56-PE/*blue*). This allows a precise NK:target ratio to be determined for each co-culture analyses (*middle row*). Note that free NK cells (*upper left gate*), free target cells (*bottom right gate*) and NK:target complexes (*upper right gate*) are all realized in the ratio scatter plots (*middle row*). Following culture and NK cell-mediated killing of target cells, dead target cells are identified by membrane disruption using the DNA intercalating dye PI (*red*). The percentage of dead target cells may be quantified (*bottom row*). Back-immunoscatter gating may be used to track live versus dead target cells via scatter properties, with the target cells manifesting altered scatter properties as they are killed (*top row*). This assay will be applied to a current study returning crewmember samples from the International Space Station

array measure cytokine levels secreted into the culture supernatant. Secreted cytokine assays measure the net level following secretion, reabsorption, and molecule breakdown. Cytokine secretion may be halted however, by adding secretion blockers during activation such as monensin or brefeldin A. This allows intracellular cytokine accumulation in the cell cytoplasm. Following such culture, it is possible to detect intracellular cytokine production by flow cytometry. For this assay, surface markers are stained first, followed by cellular fixation, and then intracellular staining consisting of anti-cytokine antibodies and a permeabilization reagent. Fixation first is required to prevent the intracellular contents from leaking out of the cell following permeabilization. Intracellular cytokine assays, by flow cytometry, measure the percentage of cells capable of being stimulated to produce a particular cytokine, not the bulk supernatant level. Cytometric bead array technology allows detection of several secreted cytokine within a single flow cytometry assay. Essentially, fluorescent bead populations with varying fluorescence intensity along a single channel are created, with each population coated with "capture" antibodies for a specific cytokine. By incubating with a common secondary detection antibody, cytokine levels may be measured independently for each cytokine along a separate channel. As measures of cytokine production by specific, positively identified cell types (intracellular), or as a measurement of bulk secreted levels of multiple cytokines, these assays have clear utility for determining the effects of spaceflight on immune system cytokine regulation. Also, this technique is problematic for some cytokines. For example, IFNg and IL-2 are readily detectable following activation; however IL-4 and IL-10 are extremely difficult. This is likely due to the normal pre-programmed secretion patterns the cells will exhibit. Examining secretion by ELISA also yields low levels for the same cytokines that are difficult to detect by cytometry. Examples of utilization follow.

Our laboratory has investigated intracellular production of IFNg and IL-2 in both CD4+ and CD8+ T cell subsets postflight for Shuttle and ISS astronauts. In general, following spaceflight crewmembers had reduced levels of both CD4+ and CD8+ T cells capable of producing both IFNg and IL-2 (Crucian et al. 2008, see also Chap. 12). Flow cytometry scatter plots showing the gating strategy and analysis technique for a representative ISS crewmember are shown in Fig. 24.3. Kaur et al. examined monocyte intracellular cytokine production of IL-1beta, IL-1ra, IL-6, and IL-8 postflight for Space Shuttle astronauts (Kaur et al. 2008). The stimulus for cytokine production was gram-negative bacterial endotoxin, known to be a powerful activator of monocytes via LPS binding to the CD14 cell surface antigen. Crewmember monocyte production of IL-6 and IL-1beta were reduced compared to controls at all three mission associated timepoints. In contrast, crewmember production of IL-1ra and IL-8 were elevated as compared to controls.

In addition to measuring intracellular cytokines for the NASA study described above, secreted production of six cytokines was measured by cytometric bead array (CBA). CBA technology analyzes multiple secreted cytokines by using capture antibodies fixed to bead populations which vary in fluorescence along a single fluorescence channel. Detection antibodies then fluoresce along a different channel, allowing a 2 × 2 cytometry dot plot with cytokine specificity along the X axis, and cytokine production along the Y axis. The array used by NASA consisted of Th1/Th2 cytokines: IFNg, TNFa, IL-10, IL-5, IL-4, and IL-2. Crewmember cells were

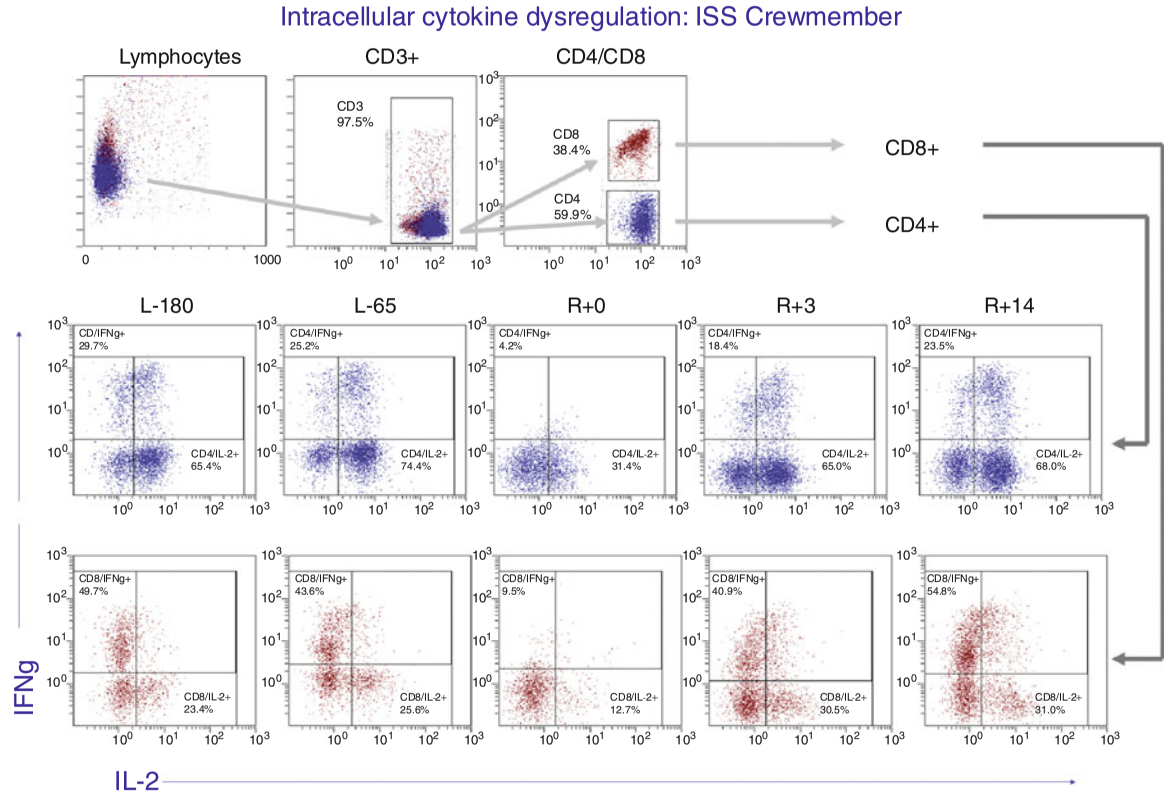
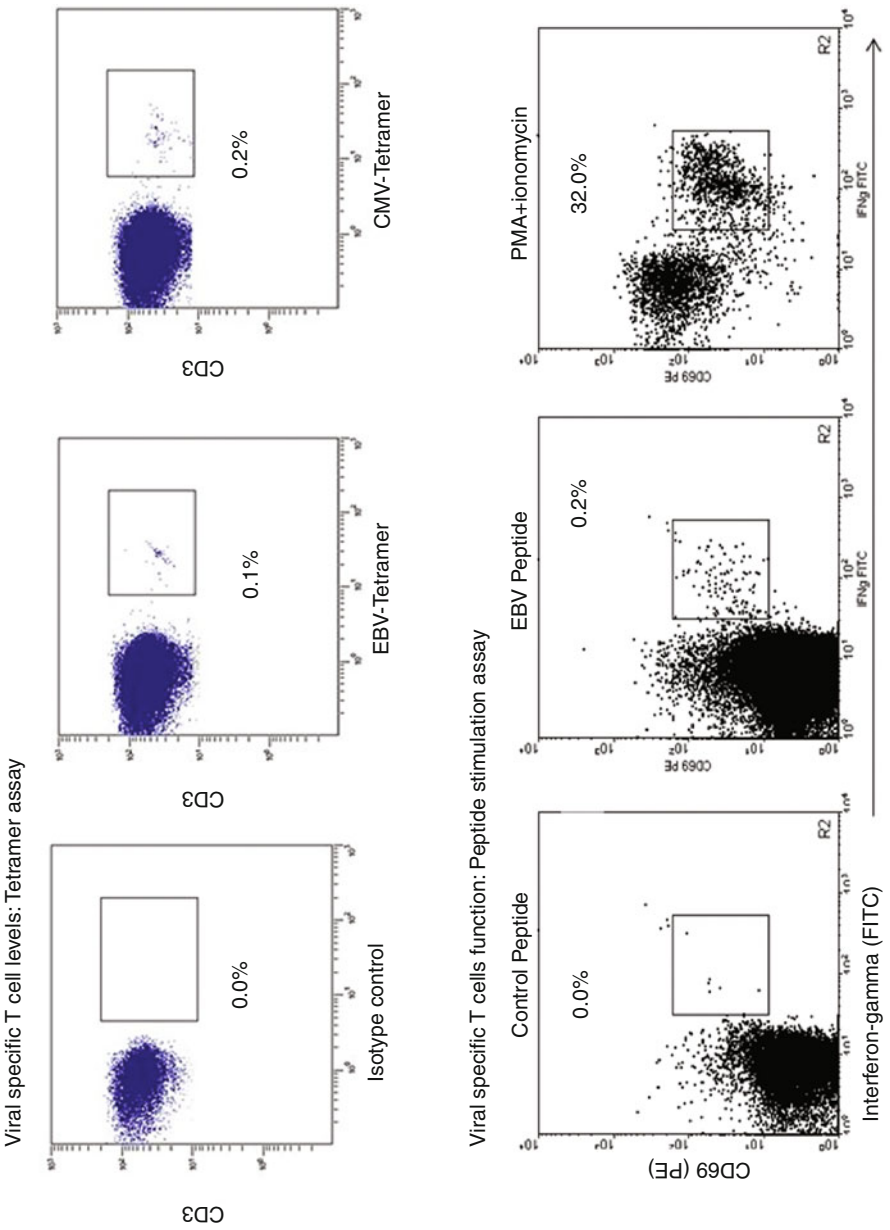


Fig. 24.3 Intracellular detection of interferon-gamma and interleukin-2 in both CD4+ and CD8+ T cell subsets by flow cytometry. Representative postflight data from a single International Space Station crewmember. Following mitogenic stimulation in the presence of monensin to halt extracellular secretion, surface markers are (in order) stained, fixed, permeabilized, and followed by staining of intracellular cytokines


Viral specific T cell levels: Tetramer assay
Isotype control
0.0%
CD3
EBV-Tetramer
0.1%
CD3
CMV-Tetramer
0.2%
CD3
Viral specific T cells function: Peptide stimulation assay
Control Peptide
0.0%
EBV Peptide
0.2%
PMA+ionomycin
32.0%
R2
CD69 (PE)
Interferon-gamma (FITC)

stimulated with either antibodies to the TCR (CD3/CD28) to stimulate T cells only, or PMA + ionomycin to stimulate all leukocyte populations. Surprisingly, there were minimal postflight alterations for most cytokines, except a reduction in IFNg for both Shuttle and ISS crewmembers (Crucian et al. 2008). Reduced production of in IFNg associated with spaceflight has been reported by other (nonflow cytometry) techniques, and may be directly related to immune loss of control of latent viral reactivation. As important indicators of immune function in general, and Th1:Th2 or pro- /anti-inflammatory biases specifically, measurements of cytokine production have significant utility for defining spaceflight-associated immune dysregulation.

24.2.4 Viral-Specific Immunity

Measurements of virus-specific T cells, either their direct enumeration from blood samples, or their functional capabilities, may have utility for monitoring spaceflight-associated immune dysregulation. There is ample evidence that reactivation of latent herpesviruses occurs during spaceflight (see Chap. 16). The reactivation of latent viruses is very likely a direct result of diminished immune function, particularly CD8+ cytotoxic T cells. Several of the viruses known to reactivate during spaceflight, EBV, CMV, and VZV, have potential direct clinical consequences should reactivation persist for very long periods of time. Should spaceflight-associated immune compromise persist for the duration of a Mars mission, crews may be at risk for these adverse clinical events. For these reasons, flow cytometry assays to detect viral-specific T cells have utility for monitoring virus-specific immunity, as current studies define the in-flight phenomenon and potentially devise strategies to monitor countermeasures validation. Stowe et al. have monitored EBV and CMV specific immunity (both number and function) during a recent postflight NASA study. This study was conducted on both short- and long-duration US astronauts, and monitored numbers of virus-specific T cells via the tetramer method, and virus-specific T cell function via a peptide stimulation assay (Crucian et al. 2009). MHC tetramers consist of multimers of MHC molecules coupled to a particular viral protein and tagged with a fluorescent label. These reagents function similar to labeled antibodies, except that they bind to antigen-specific CD8+ T cells only. For that reason, the detection population is extremely small. For CMV peptide or EBV peptide-specific T cells, normal ranges in seropositive individuals may range from 0.1% to 0.6% of the total CD8+ T cell population, making this rare-event cytometery (Fig. 24.4). As such, a gating strategy employing multiple sequential gates with populations identified by immunoscatter to thoroughly resolve target cells from debris is essential. Peptide stimulation to measure viral-specific T cell function,

Fig. 24.4 Measurement of viral-specific immunity by flow cytometry. *Top row*: detection of CD8+ T cells specific for viral-peptides via the MHC-tetramer method. *Bottom row*: determination of viral-specific T cell function by viral-peptide stimulation followed by intracellular measurement of interferon-gamma production

consists of cell culture stimulation of astronaut peripheral blood cells with a particular viral peptide and antibodies to costimulatory molecules (Crucian et al. 2009 bed rest for method, Crucian et al. 2001 peptide article). Antigen presentation of the viral peptides occurs naturally in the culture, and only viral peptide-specific T cells are activated. Cultures are processed in the presence of either monensin of brefeldin similar to the intracellular cytokine assay. Following culture, a cytometry staining protocol consisting of cell surface CD3 and CD8, and intracellular IFNg is performed to resolve activated peptide-specific T cells via production of cytokine (Fig. 24.4). When the viral peptide is a superantigen, as are several EBV peptides, concurrent staining of CD69 allows a monitoring of nonspecific superantigen activation versus legitimate viral-peptide activation (Crucian et al. 2001). The data to date indicate that, in general, numerous virus-specific T cells rise or are unchanged, whereas their functional capacity decreases (Stowe et al. 2007, HRP, Stowe et al. 2008, HRP). This at least partially explains the in-flight reactivation of latent viruses. These viral-specific assays are also being conducted as part of a current in-flight NASA study onboard the International Space Station (Crucian et al. 2010).

24.2.5 Bacterial Analysis

Flow cytometry is a versatile technique that may be applied to analyze nearly any particles in solution, provided the particles are in suspension and within the size limits for detection. The use of antibodies, fluorochromes, and membrane/functional dyes adds utility to the basic particle imaging via scattered laser light. Even bacteria, with most species within the 2–10 μm size range, may be detectable by some flow cytometers. Once successfully resolved from debris and electronic noise, there are a number of bacterial assays that may be performed by flow cytometry. Many of these have been applied to spaceflight experiments. Nickerson et al. flew *Salmonella typhimurium* onboard Space Shuttle mission STS-115, and used flow cytometry to measure bacterial suspension counts postflight (Wilson et al. 2007). SYTO-BC dye, a membrane permeant dye which stains both gram+ and gram– bacteria was used for the assay. The most recent and comprehensive of the spaceflight bacterial studies which included cytometric measurements was performed by Leys et al. In this study, *Cupriavidus metallidurans* bacterium were grown for 10–12 days onboard the International Space Station. Upon return, flown bacterial cells were compared to ground control bacterial cells using a variety of flow cytometric techniques (Leys et al. 2009). Viability, membrane potential, intracellular pH, electron transport chain function, superoxide/peroxide detection, intracellular glutathione, intracellular calcium, DNA versus RNA concentration, and intracellular esterase activity were all measured by a standard flow cytometer equipped with a 15 mW 488 nm laser. Commonly, these assays employ dyes which manifest a color change in the presence or absence of the measured condition, or presence of the measured analyte. By employing multicolor cytometry, separate channels may be used to resolve bacteria from artifactual debris, and to measure the analyte of interest. A properly tuned optical path with very low cV values is necessary to resolve bacteria on a standard flow cytometer.

24.3 Development of an In-Flight Flow Cytometer

Assuming the proven utility of some, or all, of the above described assays for monitoring immune dysregulation during spaceflight with flow cytometry, the entire development effort may be diminished without onboard monitoring hardware during exploration class space missions. Should the effect of spaceflight on immunity persist for very long periods of time, a spaceflight capable flow cytometer may be required to monitor crewmember immunity during missions beyond low Earth orbit. Unfortunately, standard commercially available flow cytometers are large, complex instruments that use high-energy lasers and require liters of liquid "sheath" fluid to operate. They also generate a significant amount of liquid biohazardous waste and require significant training to operate. Cytometers use the fluid mechanical property of "hydrodynamic focusing" to place stained blood cells in single-file (laminar flow) as they pass through a laser beam for scanning and evaluation. Many spaceflight experiments have demonstrated that fluid physics is dramatically altered in microgravity and previous studies have shown that sheath-fluid-based hydrodynamic focusing may also be altered during microgravity (Crucian et al. 2000b). For these reasons, it is likely that any spaceflight compatible design for a flow cytometer would abandon the sheath fluid requirement. The elimination of sheath fluid would remove both the problems of weight associated with large volumes of liquids as well as the large volume of liquid waste generated. It would also create the need for a method to create laminar particle flow distinct from the standard sheath-fluid-based method.

NASA has at several times investigated the possibility of an on-orbit functioning flow cytometer. In 1985, a panel convened at the Johnson Space Center generated an extensive feasibility report, but no construction was initiated. NASA has also funded several small business innovation grants for cytometer-like devices, many using microfluidics technology. Currently, Shi et al. (2009) are also working to create a microgravity compatible cell analysis device. Their device uses a portable microflow cytometer system to create a two-part leukocyte differential and leukocyte count. Leukocytes are stained with Acridine Orange and blood is pumped through a disposable microfluidics device for fluorescence sensing. Under 460 nm LED excitation, DNA (green) and RNA (red) fluorescence are collected via two PMTs. Leukocytes are counted and differentiated into lymphocytes and non-lymphocytes based on fluorescence signatures. This technology has been microgravity validated and could serve as a platform for development of a capable in-flight cytometer instrument.

To date, the closest device to a working on-orbit cytometer was likely created at the Johnson Space Center during an in-house prototyping and engineering effort. Fortuitously, a commercial cytometer was developed by Guava Technologies, which was extremely miniaturized and has eliminated the sheath fluid requirement. Although an innovative advance in technology, this instrument had primitive capabilities (2 color, 3 parameter) and was not spaceflight compatible due to fluidics handling, power requirements, sample delivery, size, and data handling. The JSC effort was designed to (1) engineer a prototype flight instrument based on the framework of the commerial off-the-shelf (COTS) cytometer; (2) perform ground-based and micro-

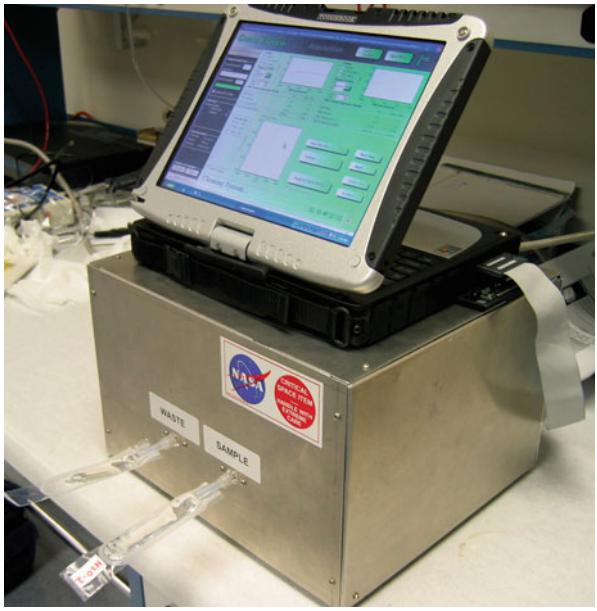


Fig. 24.5 NASA-JSC prototype flight cytometer with sample delivery WBSD units affixed to the sample delivery ports

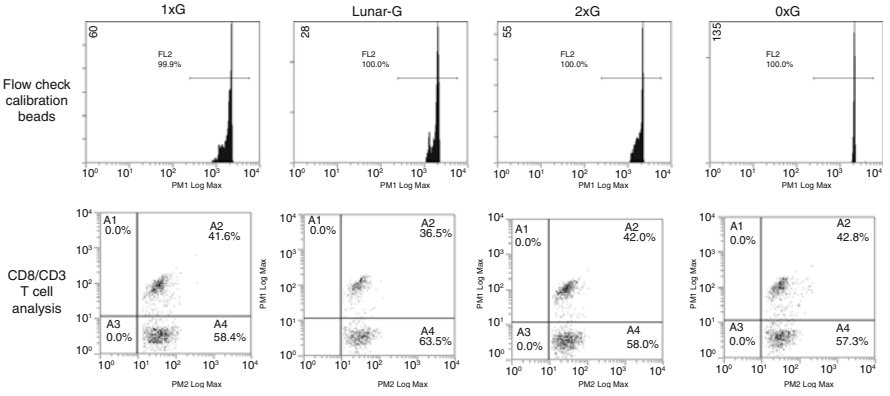


Fig. 24.6 Representative flow cytometry histograms and dot plots demonstrate performance of the prototype flight cytometer in 1xG, lunar-G, 2xG and microgravity conditions. *Top row*: assessment of flow cytometry calibration beads demonstrates detection stability and optical resolution; *bottom row*: detection of CD4+ T cells

gravity validation of the instrument; (3) design and validate a set of medical assays compatible with the prototype instrument; (4) design and validate a microgravity compatible cell staining device for sample processing that could interface with the instrument. Upon conclusion, in 2007, a miniaturized prototype was indeed developed (Fig. 24.5) and validated during microgravity conditions (Fig. 24.6), using parabolic flight aircraft. The prototype flight cytometer was found to perform cytometry adequately in microgravity, moon, mars, and even hypergravity conditions

(Crucian 2005, Crucian et al. 2006a). In addition, the Whole Blood Staining Device, designed to perform antibody staining, RBC lysis, and WBC fixation during microgravity conditions, was created. An early version of the WBSD was validated on-orbit during Space Shuttle mission STS-71 (Sams et al. 1999). Subsequently, the WBSD was further modified to successfully interface with the JSC prototype flight cytometer and allow validation of blood-to-data sample processing and analysis during microgravity conditions. Although no decision has been made regarding funding of actual on-orbit cell analysis technology for ISS (or beyond), the JSC prototype, as well as the advances in fiberoptics and microfluidics, will certainly facilitate instrument development when it is deemed that such an instrument is required.

24.4 Conclusion

As demonstrated by countless ground-based reports and the spaceflight-specific studies described above, flow cytometry is a versatile technique that has significant utility for both defining, and monitoring, spaceflight-associated immune dysregulation. This phenomenon, if found to persist during exploration class space missions, had the potential to be a significant clinical risk for crewmembers. Prolonged immune dysregulation could lead to infections, hypersensitivities, autoimmunity, malignancy, or other adverse events. The danger is likely heightened during deep space travel considering the high-energy radiation environment and lack of clinical care or return option. Cytometry-based assays, and a working in-flight flow cytometer, also in possible conjunction with other possible immune analyzers (e.g., multiplex based) using by individualized in vitro incubations platform (e.g. KUBIK), could altogether have significant utility for defining the on-orbit phenomenon, as well as the development and validation of countermeasures.

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Assessment of Radiosensitivity and Monitoring of Radiation-Induced Cellular Damage

25

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25.1 Individual Radiosensitivity

Implicit in the present day concept of radiation protection is the simplistic assumption that all individuals respond equally to radiation. However, it is well known from clinical practice as well as from radiobiological and toxicological experiments with high doses that each person reacts individually to radiation and drugs. For manned spaceflight, the individual biological responses to cosmic ray exposure are of critical concern to risk assessment for astronauts.

Interindividual differences in radiosensitivity have been reported with an increased incidence of both deterministic and stochastic effects. Several studies demonstrated that the occurrence and severity of normal tissue reactions in response to ionizing radiation are influenced mainly by genetic susceptibility. In this context, variations in radiosensitivity between individuals may be a consequence of deficiencies in DNA repair capacity. This can be caused by specific mutations or polymorphisms in DNA repair genes such as ataxia telangiectasia mutated (ATM) (Taylor et al. 1975) or Nijmegen Breakage Syndrome (NBS) 1 (Varon et al. 1998). They can also result from a combination of small variations in multiple genes (Andreassen 2005), such as those functioning in specific DNA double-strand break (DSB) repair pathways including poly (ADP-ribose) polymerase (PARP), DNA-dependent protein kinases (DNA-PKs), p53, ATM, and Rad3-related (ATR).

Ongoing research focuses on the identification of (predictive) biomarkers to determine the radiosensitivity of an individual. In the context of radiation risk assessment for astronauts, methods that can measure and/or predict individual radiosensitivity would be of great value. In the future, such assays could be used as predictive tests to identify astronauts with a greater than average risk of developing radiation-induced health effects. The identification of (predictive) biomarkers to

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distinguish between these individuals might be relevant to life not only in space, but also on Earth (radiation therapy).

25.2 Tools to Evaluate Radiosensitivity

Currently, a number of cytogenetic tests have been described and used to assess responses to radiation. In this light, peripheral blood lymphocytes are the preferred target for assessing radiosensitivity, as they are regarded as one of the most radiosensitive cell types in the human body. Biological endpoints such as chromosomal aberrations, sister chromatid exchanges, micronuclei, DNA fragmentation, and repair capacity of radiation-induced damage have been used to determine individual radiosensitivity in vitro. These methods rely mostly on previous observations that the occurrence of chromosomal aberrations is associated with increased radiosensitivity.

Different cytogenetic methods can be used for the detection of radiation-induced damage and their choice depends on several parameters (exposure duration, estimated absorbed dose, type of exposure, and time since exposure). Importantly, most cytogenetic methods can be used for rather low doses from about 0.2 Sv and upward, which is within the range of what would be encountered by astronauts on interplanetary missions.

25.2.1 Chromosomal Aberrations: The Golden Standard

Already in 1927, the subsequent Nobel Prize winner H.J. Muller discovered that X-rays could induce mutagenesis in Drosophila (Muller 1927). In the late 1930s and early 1940s it was Karl Sax who first demonstrated a linear relationship between the number of chromosomal aberrations and the radiation dose (Sax 1938). The use of cytogenetic tests to monitor the occurrence of chromosomal aberrations induced by ionizing radiation dates back to the 1960s when Tough et al. described chromosomal aberrations in the blood of patients who had radiotherapy to treat ankylosing spondylitis (Tough et al. 1960). In addition, Sparrow showed that there is a linear response between radiation dose, chromosomal aberrations, and genetic mutations (Sparrow 1961). To date, chromosomal aberrations have been widely accepted as biomarkers of exposure to ionizing radiation. Moreover, recent evidence indicates that there is an association between the frequency of chromosomal aberrations and the overall cancer risk (Distel et al. 2006; Tucker 2008) suggesting that chromosomal aberrations may be used as indicators for increased individual radiosensitivity.

Chromosomal translocations refer to the occurrence of chromosome breakage, followed by transfer of the broken-off portion, often to a different chromosome. They are usually induced by the formation of DNA DSBs and subsequent interaction of different DSBs with each other. These chromosomal aberrations are very stable, with a half-life of 6–12 years (Durante 2005) and can be transmitted by mitosis making them very suitable for retrospective biodosimetry (Awa 1997). However, the analysis of chromosomal translocations is also useful in susceptibility studies to identify genetic polymorphisms of genes involved in the individual response to

ionizing radiation (Rodrigues et al. 2005) and cancer. Currently, chromosomal translocations are used in the clinic as biomarkers for cancer risk and they have been validated as intermediate endpoints of cancer. This shows the potential of using chromosomal translocations as biomarkers for radiation susceptibility as well.

The scoring of chromosomal translocations is mostly performed using fluorescence in situ hybridization (FISH) painting. This technique uses fluorescently labeled DNA probes to mark specific regions in the DNA after which they can be visualized using fluorescence microscopy. For this purpose, metaphase spreads are usually used. However, chromosomal translocations are not always easy to detect, as radiation can induce cell cycle delay or interphase cell death. In addition, it demands considerable time to become expert in this field. Other drawbacks of the FISH technique are that it is time consuming and very expensive.

25.2.2 Micronuclei

The cytokinesis-blocked micronucleus assay is one of the most commonly used methods for the measurement of chromosome loss and breakage in nucleated cells (Fenech 2000; Fenech et al. 2003; 2010). Micronuclei are small nuclear membrane-bound structures. They originate from acentric chromosome fragments or complete chromosomes which are unable to attach to the mitotic spindle during cytokinesis, thereby resulting in exclusion from the daughter nuclei into the cytoplasm. Since micronuclei are present in cells that have completed nuclear division, they are ideally scored in the binucleated stage of the cell cycle. To distinguish between nondividing cells and cells undergoing mitosis, cytochalasin-B, an inhibitor of the mitotic spindle that prevents cytokinesis, is added to the cell cultures. As a consequence, cells that have completed one nuclear division can be identified by their binucleated appearance. This leads to cells which contain two nuclei and one or more micronuclei if chromosome breaks have occurred or the centromere is damaged (Fenech and Morley 1985). The presence of micronuclei can be detected by microscopy after staining the DNA.

Currently, this assay is being used successfully for biodosimetry after accidental radiation exposure (Fenech 2010; Fenech et al. 1997; Touil et al. 2002). Since radiosensitive cells are more susceptible to radiation-induced micronucleus formation and because micronuclei originate from mis-repair or DSBs in DNA, the micronuclei assay can be used to determine the individual response after radiation exposure. Previous studies demonstrated the usefulness of this technique to predict intrinsic cell radiosensitivity (Jones et al. 1995; Scott et al. 1996; Gutierrez-Enriquez and Hall 2003; Lee et al. 2003; Widel et al. 2001, 2003; Sprung et al. 2005).

25.2.3 Premature Chromosome Condensation (PCC) Assay

The problems that arise with the study of metaphases (e.g., radiation-induced mitotic delay) can be circumvented by directly studying interphase cell aberrations. The PCC assay is very powerful for detecting chromatin damage in G1 or G2-phase cells

and it has the advantage of being used to compare responses to different radiation types such as heavy ions or X-rays (Suzuki et al. 2006). This is in contrast to chromosome aberrations in metaphase spreads, which are not always comparable when cells are exposed to different types of radiation. The PCC method is especially suitable for studying radiation-induced chromatid breaks in the G2-phase, which is the cell cycle phase in which cells are the most radiosensitive (Wang et al. 2006). Another advantage of the PCC method is that it can be used for dosimetry purposes, especially after exposure to high doses (>5 Gy) (Balakrishnan et al. 2010).

PCC occurs when mitotic cells, containing condensed chromosomes, fuse with interphase cells causing the interphase cells to produce condensed chromosomes prematurely. Although the initial protocol was very laborious and did not hold much promise to become a widely used method, a study by Gotoh and Asakawa (Gotoh and Asakawa 1996) showed that it was possible to induce PCC in G2-phase lymphocytes using the protein phosphatase inhibitor okadaic acid. Later, other protein phosphatases were also applied (Durante et al. 1998), thereby significantly enhancing the suitability of the technique. To date, the PCC assay is the preferred technique for biodosimetry of high doses (Balakrishnan et al. 2010).

25.2.4 Comet Assay (Single Gel Electrophoresis)

The comet assay, also known as the single-cell gel electrophoresis assay, is a sensitive method for the detection of DNA damage and repair in individual cells (Olive et al. 1990; Singh et al. 1988, 1995). In this assay, cells are embedded in a thin layer of low melting point agarose on a microscope slide. Following lysis of the cells, all membranes and soluble cell constituents are removed, except for DNA. When subjected to an electric field, broken DNA fragments migrate out of the cell in the direction of the anode appearing like a "comet" with a distinct head and tail. The size and shape of the comet and the distribution of DNA within the comet correlate with the extent of DNA damage (Fairbairn et al. 1995). For many years now, the comet assay has been utilized to study DNA damage induced by ionizing radiation (Alapetite et al. 1999; Fairbairn et al. 1995).

25.2.5 γ–H2AX Focus Assay

Upon DNA DSB induction in mammals, the histone H2A variant H2AX becomes rapidly phosphorylated at serine 139 in a region spanning several megabases around the initial lesion. The phosphorylated form of H2AX, termed γ-H2AX, facilitates the recruitment of other DNA repair factors to the damaged sites (Paull et al. 2000) and has an anchoring function to retain the broken chromosomal DNA ends in close proximity (Bassing and Alt 2004). As a result of the recruitment of DNA repair factors, a so-called focus is formed which can be easily visualized using light microscopy (Rogakou et al. 1998, 1999). As there is one focus formed per DSB, the number of DSBs can be directly determined from the number of foci that are present shortly after the induction of DNA damage.

The phosphorylation of H2AX occurs at a conserved carboxyl-terminal Ser-Gln-Glu (*S*QE) amino acid sequence and is catalyzed by members of the phosphoinositide 3-kinase (PI3K) family, such as DNA-PK catalytic subunit ATM, and ATR (Rogakou et al. 1998; Paull et al. 2000; Bassing et al. 2002). The phosphorylation status of γ-H2AX is under tight control of the kinase activity of the above mentioned protein kinases, as well as the phosphatase activity of protein phosphatases such as WIP1/PPM1D (Cha et al. 2010). Another way of removing γ-H2AX, after DNA damage has been repaired, is by histone exchange. It has also been shown that the mechanism of γ-H2AX phosphorylation is dependent on the type of genotoxic stress. For example, at the site of ionizing radiation-induced DSBs, the phosphorylation of H2AX occurs mainly by ATM, whereas after UV irradiation the mechanism depends on the cell cycle and can occur in the absence of DSBs (Cha et al. 2010).

It has been demonstrated that radiosensitive phenotypes retain H2AX foci for a longer period of time compared to radioresistant cell strains. In this way, measurement of repair kinetics of DNA DSB by scoring H2AX foci is a good candidate approach for analyzing cellular radiosensitivity (Rube et al. 2008; Werbrouck et al. 2010).

25.3 Emerging Technologies for High-Throughput Screening

To date, classical cytogenetic measurements such as dicentrics and chromosomal translocations have been used mostly to detect chromosomal aberrations in peripheral blood lymphocytes of astronauts returning from long-term space missions (Cucinotta et al. 2008; Fedorenko et al. 2001; George et al. 2001; Obe et al. 1997; Testard et al. 1996). These studies have shown that the number of chromosomal aberrations significantly increased during the mission, although there were large interindividual differences, which could not be attributed to the measured dose. This variability could be a consequence of differences in radiation sensitivity, but the experimental uncertainties were so large that the result can also be explained by statistical fluctuations (Durante 2005) because the doses to which the astronauts were exposed were close to the sensitivity thresholds of the assays (Cologne et al. 1998). Another disadvantage of most of the classical cytogenetic assays is the fact that they are labor-intensive, since they require the scoring of thousands of mitotic cells (metaphase spreads) which cannot be performed fully automated. In the next paragraphs, we will review some emerging technologies which might be useful for high-throughput screening of interindividual differences in radiosensitivity.

25.3.1 Genetic Profiling

Intrinsic radiosensitivity is correlated with the ability of the cell to detect and repair DNA damage (Hennequin et al. 2008), which is the ultimate factor for cell survival. To deal with radiation-induced DNA damage, cells have developed complex responses that rely on activation of genes that are involved in DNA damage repair and cell cycle arrest. However, when the cell damage is too severe, cellular apoptosis can be induced as well. Thus, individuals who have a genetic dysfunction of

certain genes that are important for the DNA damage response display hypersensitivity toward ionizing radiation. In this context, not only the quality but also the quantity of changes in gene expression may differ greatly between individuals and contribute to the individual differences in response to radiation (Smirnov et al. 2009). For instance, in a screen of the radiation response between unrelated individuals and monozygotic twins it was shown that there was a very strong heritable component for the transcriptional response of the p53 target genes FDXR and CDKN1A to ionizing radiation. The variability in their postirradiation expression levels was much smaller amongst the monozygotic twins than the unrelated individuals (Correa and Cheung 2004).

Over the past few years, several studies have demonstrated the usefulness of gene expression signatures for biodosimetry purposes (Brengues et al. 2010; Dressman et al. 2007; Kabacik et al. 2010; Meadows et al. 2008; Paul and Amundson 2008; Pogosova-Agadjanyan et al. 2011). Most of these studies have investigated radiation responses to doses between 0.5 and 10 Gy in peripheral blood lymphocytes, and could demonstrate that the use of gene expression signatures is especially valuable below 5 Gy. To our knowledge, virtually no studies have been published so far on the use of gene expression signatures for doses below 0.5 Gy. Unpublished data from our group indicate that gene expression profiles can be used to predict doses below 0.1 Gy of X-irradiation. From our observations we can conclude that the genes most appropriate for biodosimetry are those involved in the p53-regulated DNA repair, cell cycle, and apoptosis pathways, similar as to what would be expected for individual radiosensitivity. However, in contrast to gene expression signatures for individual radiosensitivity, genes that are useful for biodosimetry should ideally exhibit dose-dependent changes in expression with very little variation depending on age, gender, time, cell type, and/or other interindividual confounding factors (Pogosova-Agadjanyan et al. 2011).

25.3.2 Epigenetic Profiling

Besides individual differences in gene expression, differences in epigenetic marks may also influence the individual response to ionizing radiation. The term epigenetics has nowadays many different meanings. One classical definition is that epigenetics refers to changes in gene expression that do not involve changes in the DNA sequence but are nevertheless inherited (through mitosis and possibly meiosis) (Holliday 1987). Two classical epigenetic mechanisms are DNA methylation and posttranslational histone modification, both of which mainly affect gene expression by altering the chromatin structure, thereby making genes more or less accessible for transcription. Other epigenetic information carriers that have been proposed include transcription factors, prions, small RNAs, and chromatin structure (Kaufman and Rando 2010).

DNA methylation is the most widely studied epigenetic modification in humans (Portela and Esteller 2010). It involves a covalent deposition of a methyl group at the 5′ position of a cytosine ring, which occurs mostly in the vicinity of a CpG

dinucleotide. The transfer of the methyl group is a replication-dependent reaction catalyzed by DNA methyltransferases (DNMTs), which are present at the replication fork during the S-phase (Taby and Issa 2010). The CpG dinucleotides tend to cluster in so-called CpG islands which are often located in promoter regions of genes (about 60% of the human gene promoters are associated with CpG islands). In a normal differentiated cell, most promoter CpG islands are unmethylated whereas the CpG islands that are distributed across the genome – mostly associated with repetitive elements – are methylated. Methylation of CpG islands is generally associated with gene silencing via various mechanisms such as recruitment of methyl-CpG-binding domain proteins, recruitment of histone-modifying and chromatin-remodeling complexes, or by precluding the recruitment of DNA-binding proteins (e.g., transcription factors) to their target sites (Portela and Esteller 2010).

Cancer cells display genome-wide aberrations at the level of DNA methylation, including global hypomethylation and hypermethylation of specific promoters, for example, of tumor-suppressor genes, which lead to selective growth advantages compared to neoplastic cells (Taby and Issa 2010). In this sense, profiling of methylation markers can now be used for classification of cancer types (Jones and Baylin 2007). In a recent study (Kim et al. 2010), genome-wide DNA methylation profiling was used for analyzing differences in radiosensitivity of cancer cell lines. They found that more than 1,000 genes (7.5%) were differentially methylated between a radiosensitive and a radioresistant human non-small cell lung cancer cell line. Several of these genes were analyzed further and were shown to be important for cell survival in response to ionizing radiation (Kim et al. 2010). Although this kind of analysis is still in its infancy, it may be expected that such methodologies will be applied to assess individual radiation responses in cancer patients, as well as radiation-exposed workers such as astronauts.

25.3.3 Multiple Protein Expression Profiling

Although changes in gene transcription can serve as good candidate biomarkers, protein changes may be more relevant for determining the underlying mechanisms of individual radiation responses. In this regard, two major areas which can contribute to the discovery of new biological markers of radiation sensitivity are in the fields of proteomics and multiple protein expression profiling. Differences in protein levels can occur between radioresistant and radiosensitive individuals, and therefore protein signatures might also have a predictive value. Recent advances in proteomics might allow the identification of proteins associated with radiosensitivity (Smith et al. 2009; Turtoi et al. 2010).

Cytokines are soluble proteins that are secreted by specific cells of the immune system. They are signaling molecules that are used extensively in cellular communication. Different cytokines act together to orchestrate immune responses. Advances in techniques such as the Luminex system® have made it possible to simultaneously analyze multiple analytes within a single sample (Nolan & Mandy, 2001). This high-throughput technique can also be useful to decipher complex radiation-induced

responses. The technique uses microspheres that are labeled internally with fluorescent red and infrared dyes. By varying the ratio of the two dyes, bead sets of up to 100 different beads can be created, each with a unique spectral signature, determined by its red/infrared mixture. For protein analysis, each bead set can be coated with antibodies of interest, allowing the simultaneous analysis of up to 100 proteins. Currently, the Luminex FLEXMAP 3D ® system uses a combination of three different dyes, which increases the amount of different bead sets to 500. The analysis of the sample is based on a capture sandwich assay format. After incubation of the sample with the beads, detector antibodies are added, followed by an addition of a third fluorescent reporter reagent. The spectral properties of the beads are then monitored by the Luminex instruments. Upon excitation by a laser, the emission wavelength can be used to classify each bead into the appropriate bead set, while the intensity of the fluorescent signal emitted by the reporter fluorophore is proportional to the amount of protein bound to the bead.

Using the Luminex system, Blakely and colleagues developed a radiation-sensitivity bioassay consisting of proto-oncogenes such as ras-p21, raf-1, and DNA repair p21/Waf1/Cip (Blakely et al. 2005). Another study demonstrated significant levels of cytokines, including TGF-β1, IL-6, KL-6, surfactant proteins and IL-1, on predicting radiation-induced lung toxicity (Kong et al. 2008). Related to cytokine expression, a study by Fujimori demonstrated ionizing radiation-dependent increases in chemokines (Fujimori et al. 2005). The value of cytokine dynamics for predicting individual radiation sensitivity is currently being investigated.

Besides profiling cytokine changes to detect interindividual differences in response to radiation, the use of phospho-specific detection antibodies in multiplex assays might have potential as well. It is well known that in signal transduction pathways, the activation of the mediators of the pathway involves, in many cases, the phosphorylation of sites in their tertiary protein structures. Thus, interindividual differences in, for example, the phosphorylation status of p53 in response to ionizing radiation might have a predictive value (Shrivastav et al. 2008). Besides phosphorylation, other posttranslational modifications may also be indicative of individual differences in radiation response.

25.4 Conclusion

For manned spaceflight, the biological effects induced by cosmic ray exposure on the immune and other organ systems are of critical concern to risk assessment for astronauts. In this light, the increased risk of cancer associated with radiation exposure is widely considered to be the main obstacle to interplanetary travel (Cucinotta and Durante 2006; Durante and Cucinotta 2008). Shielding of the astronauts from space radiation is therefore a very important protective measure. Material shielding may only be partially effective against cosmic radiation in certain energy ranges, but may actually make the problem worse for some of the higher energy rays, as more shielding induces an increased amount of secondary radiation. Therefore, other protection measures such as onboard biodosimetry, or therapeutic measures, should

also be considered, although their effectiveness in deep space is not yet established. Currently, several radioprotectors are available that can prevent and/or reduce radiation-induced health effects by enhancing the body's natural capacity to repair cell damage caused by radiation (reviewed in Dziegielewski et al. 2010). Another possible preventive measure could be to include the individual's radiation resistance to the induction of early and late radiation effects in the medical assessment of the mission crew applicants. This has to be complemented by the provision of adequate onboard biodosimetry tools to guarantee the best possible diagnostics and personalized care in-flight. However, their effectiveness in deep space is not yet established.

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Part V

Preventive and Therapeutic Strategies

Considerations for Preventive and Therapeutic Strategies

26

Jean-Pol Frippiat, Martina Heer, and Alexander Choukèr

Astronauts have experienced altered immune function and increased vulnerability to infections during spaceflights dating back to the Apollo and Skylab missions (Kimzey 1977) (see for details Chaps. 9–16) and one might consider robotic missions to circumvent any further risk to humans on mission to space. However, even though robotic mission will undoubtedly precede and further accompany manned exploration mission ("human robotic exploration teams"), the capabilities of man to support space exploration missions, *as an intelligent tool*, with his capacity to estimate situation by previous experiences, to change plans considering complex conditions, develop and communicate ideas, not just data, and react intuitively makes him overall irreplaceable for now. These manned or manned-robotic missions could be "missions to the surfaces of solid bodies whose surface conditions are not too hostile for humans" and hence in a foreseeable time frame, man may voyage and "realistically explore the surfaces of the Moon, Mars, Phobos and Deimos, and some asteroids" (Committee on the Planetary Science Decadal Survey 2011).

Maintaining crew health requires also the development of efficient measures to prevent from and combat the deleterious effects of spaceflight on the immune system. This is an area that should be considered more thoroughly before we undertake prolonged space voyages (Brumfiel 2007; Heppener 2008). These preventive or even therapeutic measures will likely range and include alike psychological (Chap. 27), physical exercise (Chap. 28) as well as pharmacological (Chap. 29) and nutritional

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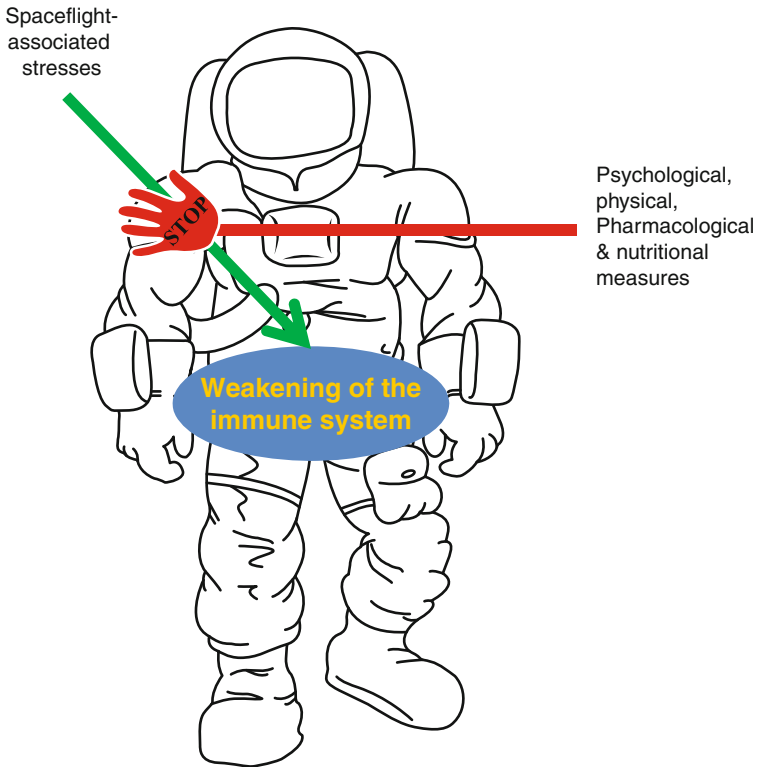

Fig. 26.1 Mitigation of unfavorable immune alterations by selected approaches. Developing psychological, physical, pharmacological as well as nutritional measures to prevent from spaceflight-related immune alterations or to limit their extent are important for astronauts but also for people presenting a weak immune system on Earth because of infection, aging and/or chronic stress exposure

(Chap. 30) approaches (see Fig. 26.1). Consequently, several scientific investigations have been conducted to investigate the role of psychological measures to mitigating the likelihood of psychological problems among the crew during extended space missions, the effectiveness of physical exercise to maintain and ameliorate mood, to maintain adequate immune allostasis, and to hereby reduce health risks under those extreme conditions of life. Moreover, laboratories have undertaken studies to understand the impact of malnutrition and nutritional and pharmacological approaches to combat the deleterious effects of spaceflight on the immune system. Despite good strategies, not all approaches were successful. This was, for example, the case when pegylated interleukin-2 (IL-2) – a covalent attachment of polyethylene glycol polymer chains to the cytokine IL-2 – was tested on rats subjected to two 8-day spaceflights (Chapes et al. 1999). On the other hand, malnutrition often reported in astronauts and cosmonauts (Smith et al. 2009) may exacerbate immune function alterations in long-term spaceflight. The most promising nutritional and

pharmacologic approaches deduced from human investigations as well as from experimental setting are presented in the following chapters. However, this overview can neither cover all detailed strategies of combined action of tentative countermeasures, nor tentative concepts of immune modulation by boosting immunity through vaccinations in space. This last strategy may be less efficient than originally expected since a recent report showed that somatic hypermutation, which diversifies antibody-binding sites to improve their quality occurs in space following immunization but at a lower frequency (Bascove et al. 2011). Chapters 29 and 30 inform and strengthen the need, despite the increasing knowledge in this field, of an appropriate understanding of the complex nutrition–immune interactions and of finding an approach to "the right" balance for nutrient supply or even supplementation. Moreover, it is also important to understand the need, timing, and efficiency in space for both, nutritional and pharmacologic interventions triggered by adequate immune read-out parameters. Achieving a better understanding of stress challenges during space missions and the description and testing of an efficient and balanced set of nutritional and pharmacological together with physical and psychosocial measures will open up new preventive and therapeutic tools helpful to counterbalance immune dysfunctions encountered in Space and on Earth, such as those induced by aging or acute and chronic stress exposures (Godbout and Glaser 2006).

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Psychological Countermeasures

27

Gro Mjeldheim Sandal and Gloria R. Leon

27.1 Introduction

Psychological research has been recognized as a major part of understanding challenges associated with human space exploration. Long-duration missions may involve chronic exposure to many stressors that can negatively impact health, performance, and even safety. A growing body of research on crews working for extended periods in different kinds of isolated, confined environments reveals the existence of psychological and performance problems in varying degrees of magnitude. Psychological reactions have included depression, emotional lability, fatigue, sleep difficulties, decrements in cognitive functioning, psychosomatic symptoms, irritability toward crewmates, and/or mission control staff, and a considerable decline in vigor and motivation (Kanas and Manzey 2008).

In the book "Dragonfly: NASA and the Crisis Aboard Mir," Bryan Burrough (1998) described many of these reactions among astronauts and cosmonauts who served aboard the Russian space station. For example, at one stage, communication between one of the NASA astronauts and the ship's cosmonaut commander was so limited as to be almost nonexistent. A veteran astronaut returned from Mir suffering from exhaustion and depression, blaming both on NASA's lack of ground support. Clashes of cultures were evident within the crew as well as among the personnel in the mission control particularly in terms of handling of safety sensitive information.

Together with other spaceflight factors, such as microgravity, exposure to prolonged stress can alter physiological responses, including the immune system, and thereby pose increased health risks for crew members during long-duration

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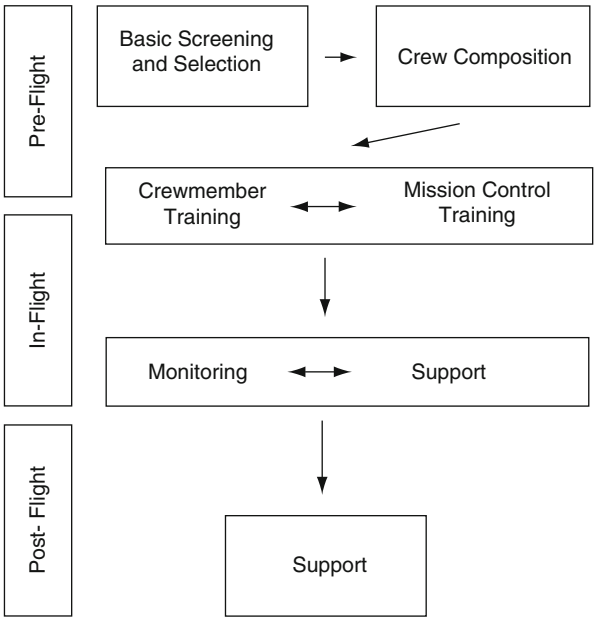

Fig. 27.1 Elements of psychological countermeasures (from Kanas and Manzey, 2008. Reproduced with permission)

space flights (Choukèr et al. 2001). The purpose of this chapter is to discuss countermeasures that may act to reduce or even prevent psychological and interpersonal problems during long-duration missions, and thus have a positive impact on the resilience of crew members. Kanas and Manzey (2008, p. 161) define psychological countermeasures as including "all actions and measures that alleviate the effects of the extreme living and working conditions of space flight on crew performance and behaviour." They distinguished between two complementary approaches. One approach focuses on the accommodation of the working and living conditions during space missions to the psychological capabilities and needs of humans, for example, ergonomics, work design, and work-rest scheduling. The second approach focuses on adapting crew members to the psychological vicissitudes of space missions. The latter can be achieved by psychological selection of astronauts, crew composition based on interpersonal compatibility, pre-flight psychological training, and psychological support to individual astronauts and crews while they are on-orbit as well as to their families. In addition, postflight support activities assisting with the re-adjustment of astronauts to life on Earth after a space mission is also a psychological countermeasure, since they help to prevent adverse psychological aftereffects of space flight. As the former approach typically represents multidisciplinary efforts and approaches, the focus of this chapter will be on countermeasures falling into the latter category (see Fig. 27.1).

A review of the history of human spaceflight reveals large differences between agencies in the extent to which psychological challenges have been officially recognized and addressed by appropriate countermeasures. From the beginning of the Soviet/Russian space program, psychological countermeasures have been an important

element. In contrast, attention to psychological factors in research and operations were not a high priority for NASA over a considerable period of time (Harrison and Fiedler 2011). Many observed that the NASA culture discouraged questions about the behavioral health of astronauts since they were assumed to have the "Right Stuff". Recently, the importance of psychological factors in mission success has become more salient as a result of experiences during the Russian/American MIR/Shuttle and the International Space Station (ISS) programs. Psychological countermeasures are now being used by all agencies in current operations on ISS and they will be an indispensable factor during future exploratory space missions that go beyond Earth's orbit.

27.2 Selection

Selection is the process of identifying from a pool of applicants those who are best fit to work as astronauts. At the individual level, the objective of selection strategies is twofold: to eliminate unfit or potentially unfit applicants, and to select from otherwise qualified candidates those who will perform optimally. A distinction is therefore made between "select-out" and "select-in" criteria.

***Select out*:** The select-out process most often involves psychiatric and psychological screening procedures designed to reduce the likelihood of psychological dysfunction and impaired performance. Psychiatric screening interviews and standardized tests (in particular, the Minnesota Multiphasic Personality Inventory) have been used by the U.S., Russian, and European space agencies. Some mental disorders, for example, certain types of depression and anxiety disorders, as well as liability for schizophrenia have a considerable genetic component that might be detected by inquiries about family psychopathology as part of the interviews. As the field of genetic testing and other psychometrically based predictors of dysfunction develop over time, this information will be used in screening, selection, and follow-up evaluations.

A challenging issue in selecting crews for future long-duration and planetary missions is the prediction of the later development of psychopathology in initially healthy individuals (Leon 2009; Sandal and Leon 2011). The onset of a serious disorder during a space mission would affect not only the disabled person, but would also have a deleterious impact on the performance of the full crew, their safety, and potentially could jeopardize the mission. Passing a psychological/psychiatric screening examination at initial selection does not necessarily predict the absence of psychological/behavioral problems that might occur at a future time. Therefore, it is imperative that assessment of the psychological fitness of the astronauts is carried out on a regular basis and in particular before and after participation in space missions.

***Select in*:** Establishing assessment tools to select the best individuals for the astronaut corps from a pool of healthy applicants also represents a challenge. The psychological *select in* process focuses on identifying candidates who, according to their operational aptitudes, attitudes, and personality characteristics, appear to be best suited for spaceflights. Kanas and Manzey (2008) have pointed out that current

attempts to define select-in factors for astronaut selection have been based primarily on expert judgments, which may seem plausible but usually lack scientific validation. To date, the absence of formal criteria for assessing astronaut performance and the limited research opportunities for longitudinal studies have made it difficult to evaluate the predictive power of personality traits. However, relevant research has been conducted during shorter missions and in analog environments on Earth that entails some similarities to the conditions that astronauts experience in space. For example, a study of military units undertaking survival training identified strong associations between cortisol levels and personality traits that have been suggested as criteria for astronaut selection (Sandal et al. 1998). The stress hormone cortisol may have a negative impact on performance through the generation of anxiety and fatigue states, but may also affect health parameters though suppression of the immune system. Such research represents steps in providing empirical evidence that can be applied in identifying the "Right Stuff" in astronauts, and assist in defining scientifically sound select-in criteria.

NASA historically has focused its selection process on select-out procedures based on psychological tests and psychiatric interviews. The Soviet/Russian program, in addition, has included careful assessment of psychophysiological responses in induced high stress situations. Comparing the different select-in approaches applied in Russia, Japan, Canada, Europe, and the USA, there seems to be overall agreement that at least the following aspects need to be considered in evaluating psychological fitness for spaceflight: motivation, relevant biographical experiences, cognitive and psychomotor capabilities, stress-coping, interpersonal and team-work skills. Despite this consensus, differences in the national and organizational cultures of the space agencies are probably reflected in the choice of methods used as part of the selection process.

For the European Space Agency (ESA), representing 18 member states, an important consideration is that psychological selection methods should not favor or disfavor applicants from certain countries or language groups. In the selection round performed in 2008, a total of 8413 valid applications were received. After psychological, medical and professional screening, six new ESA astronauts were selected. From an assessment point of view, assuring a fair and accurate evaluation in a multicultural and multinational pool of applicants raises a number of challenges. One issue is whether candidates should complete the same or different language versions of a psychological measure. Another issue is whether the results from multinational candidates should be compared using a common norm or the local norms of their countries. At present, there are no definite answers to these questions.

27.3 Crew Composition

Psychological guidance in crew composition involves consideration of the *interpersonal compatibility* of crew members assigned to specific missions. Interpersonal compatibility is a concept that describes the long-term interaction among individuals in relation to ease and comfort of communication and behavior. It assumes

greater importance with increasing mission duration as long-term social monotony and confinement are likely to increase the risk for interpersonal tension that may negatively impact on psychological health and the ability of crew members to collaborate efficiently. While no general theory of interpersonal compatibility exists within psychology, research on groups operating in confined and isolated environments suggests that compatibility is determined by similarities in personality characteristics, needs, values, and motivation (Sandal et al. 1995, 2011); expectations about specific work performance roles; and congruence of abilities with task requirements (Leon et al. 2011).

An important question is the selection of the most efficient tools for assessing interpersonal compatibility. Because congruent personality traits seem to play an important role, comparing individual scores on standardized personality questionnaires represents one approach. However, few personality tests have been validated for such purposes. Russian space psychologists have based their compatibility assessments in part on psychophysiological testing undertaken during group exercises (e.g., the "Homoestat," Gazenko 1980). Yet, very little empirical research has been published about these methods in the accessible literature, and the theoretical basis of at least some of these approaches has been called into question (Manzey 2003; Santy 1994). At present, careful observation of group interactions that may include behavioral exercises combined with mutual peer ratings seems to be the most accepted basis for assessment of interpersonal compatibility. This approach has successfully been implemented in crew selection for several space simulation studies in which ESA has been involved.

Since the beginning of long-term flights in the Salyut 6 orbital station, the Russian space program has strongly emphasized interpersonal compatibility of crew members. In these earlier days, crew composition was decided well in advance of the mission, which allowed for observation of the interactions of crew members as they trained together on Earth. In the MIR program, the addition of astronauts from foreign agencies, whose flight assignment was not under the full responsibility of the Russians, added a new dimension of complexity to maintaining crew compatibility. Suddenly, the procedure of rotating one crew member from a flight no longer allowed for the well-established practice of replacing the entire prime crew with the backup crew if the prime crew was found to be interpersonally incompatible. Within the ISS program, the shift from three to six crew member operations – with two teams of three coming onboard every 3 months, and the associated required handover of leadership – has injected a change. As a result of these constraints, it is hardly feasible to compose a psychologically guided crew based on observational data. Also, in practice, each partner agency nominates its own candidates for certain missions, and social, political, and cultural forces will always be contributing factors. On the other hand, it will be even more important for interplanetary space missions to use a psychologically informed method of composing the crew. While this already has been ascribed some significance for orbital space missions (Gazenko 1980; Manzey 2003), it will become a pivotal element for missions involving high levels of crew autonomy, such as a mission to Mars.

27.4 Pre-mission Training

Psychological training programs aim to prepare the crew for individual, psychosocial, and cultural challenges they may experience during the mission. Promoting the ability of crew members to cope with their individual reactions is one important aspect of pre-mission training. For example, learning principles for optimizing the quality of sleep ("sleep hygiene" and relaxation techniques) may be of great value based on the frequency of sleeping problems reported by crews in space. Given the psychosocial demands of long-duration mission and the interdependence between crew members for task accomplishment, conflict management and team building activities are also important aspects of pre-mission training. These activities aim to build an optimal crew cohesion, which is known to have a strong influence not only on the impact on performance but also on the psychological resilience of group members in stressful situations.

Of special concern for long-duration missions to Mars and beyond is the retention of optimal cognitive and perceptual-motor skills over long periods without "on-the-job" practice. To compensate for this problem, crew members and mission control personnel may receive computer-based psychosocial education refresher courses to remind them of key issues discussed prior to launch. Such programs are currently being tested during a Mars mission simulation study at the Institute of Biomedical Problems (IBMP) in Moscow.

The Russian, American, and European space programs all offer team building and group activities as part of pre-mission training. However, published information about the contents and methods of training has been sparse. According to Santy (1994), Russian psychological training focused mainly on stress management and the familiarization with stressful events during field exercises like survival training, parachute jumping, or periods in isolation chambers. American approaches during the MIR/Shuttle program were limited to theoretical briefings to crew members and their families about psychiatric and psychological issues of long-duration spaceflight (Ritsher 2005). Now that the Russian, European, and American space agencies are equal partners on joint projects such as the ISS, they are faced with challenges related to collaboration of multinational teams representing different national and organizational cultures. This situation has increased the need for empirically tested standardized training programs. Recently, a working group that included experts and specialists from different disciplines and all major ISS international partners was tasked to develop a so-called "ISS Human Behavior and Performance Competency Model" (Tomi et al. 2008). This model was developed for implementation as a prerequisite to ISS flight assignment and other international long-duration missions. For example, the guidelines call for the early and frequent exposure of astronauts and cosmonauts to relevant ISS languages and cultures through professional trips and full-immersion language courses. This training aspect has also been emphasized by crew members who have experience with multinational missions.

Crew members and mission control personnel have emphasized that prelaunch psychosocial training should involve key members of both groups to prepare them for coping with psychological issues occurring during long-term missions and to

establish mutual trust. A frequent observation during actual as well as simulated space missions is that crew members express frustration about lack of empathy from mission control personnel (Gushin et al. 1997; Sandal et al. 1995; Kanas et al. 2000). Although this tendency to some extent has been interpreted to be a displacement of tension, it is extremely important that the space crew and the Mission control are able to understand each others' perspectives. When training for a specific ISS mission, joint training of crew members and flight control teams is often not possible due to scheduling constraints. However, it is still possible to perform joint training at an earlier stage, namely, during basic and preassigned crew training. Exposing the crew and mission control teams to the same behavioral training, using similar case studies, situations, and role-play scenarios creates a basic, common culture and language for all operational personnel throughout the program.

27.5 In-Flight Support

Countermeasures necessary for long duration space missions can be described as targeted at two interrelated actions. The first involves monitoring the mental, emotional, and psychophysiological state of crew members, prevention of and intervention when problems are evident; the second action involves several in-flight support measures to prevent feelings of monotony, boredom, and social isolation. In addition, countermeasures are needed to enhance the positive experiences of spaceflight, as well as mitigating interpersonal tensions among crew members and between space crews and mission control.

The purpose of behavioral *monitoring* is to identify the possible emergence of psychological dysfunction among crew members. Early, accurate detection of even mild symptoms and the rapid implementation of countermeasures to deal with these problems are extremely important in long-duration space missions because of the extraordinarily high level of sustained performance required for safety and mission success. Methods for psychological monitoring are dealt with in Chap. 19.

Basic elements of *in-flight support* involve helping astronauts to stay connected to life or Earth. In-flight support has been carried out in Russia since the earliest days of human spaceflight. Activities have included frequent communication with families via two-way video, radio, and television programs, and delivery of gifts, letters, and favorite food via the progress cargo ship. In addition, the arrival of visiting astronauts and cosmonauts on MIR and ISS has helped break the monotony and provided stimulation and assistance in performing mission activities (see Kanas and Manzey 2008). Based on the Russian experience, a similar system has been established by NASA for its space station support activities (Sipes and Van der Ark 2005).

The spaceflight experience affects individual astronauts differently. The enhancement of individually tailored leisure time activities that takes into account changing interests and needs over the course of the mission is an important element of psychological support systems. As an illustration, clear differences in leisure pursuits were described among crew members on MIR: Shannon Lucid read books; John Blaha watched videos, and Andy Thomas sketched. The U.S. astronaut Jerry

Linenger (2002) published a book "Letters from MIR: An astronaut's letters to his son" that he wrote during his period on the ISS.

Family separation and concerns about beloved ones on Earth may represent a significant source of mood and morale change in people who work in extreme environments (Palinkas 1992). Nonetheless, some astronauts indicate that they are able to set aside these concerns during training and flight through so-called "compartmentalization." Astronaut Daniel Tani was living on the space station, with no way to get back to Earth quickly, when his mother died in a car crash in 2007. He declined his bosses' offer to cut his workload and continued to work productively for the rest of his stay in orbit. Recently, astronaut Mark Kelly decided to continue as Commander of a space shuttle mission even as his wife recovered from being shot. Providing psychological and social support to families during the mission can be helpful in maintaining the crew members' concentration on the objectives of the mission by relieving them of considerations about possible problems at home and feelings of responsibility.

Families need to be coached in interacting with their in-space family member and be prepared for possible psychological changes during and following the mission. Regularly scheduled private psychological conferences between crew members and ground consultants, using two-way audio or video transmissions, is routinely offered to crew members on the ISS. For this countermeasure to be effective, it remains essential that information is treated in a highly confidential manner. During international flights, crew members should be given the opportunity to speak with consultants who share the same cultural background and in their native language. On future missions to Mars, access to expertise on Earth will become more limited as most of the mission will occur at so great a distance that the communication delay will impair the effectiveness of the consultation. These communication challenges will mandate the use of methods for self-assessment to identify and deal with psychological/psychiatric or interpersonal disorders. Such systems are currently being tested during simulated and actual space missions. For example, computer-interactive instruction programs for the treatment of depression, anxiety, and stress management, and conflict resolution are currently being developed by investigators funded through the NASA-supported National Space Biomedical Research Institute. The goal of these programs is to enable astronauts to overcome emotional and behavioral problems through teaching modules they can access in private.

Psychoactive drugs always need to be part of the onboard medication kit as a countermeasure for emergency and chronic psychological/psychiatric problems developing during long-duration missions. However, the choice of specific mood/behavioral altering medications needs to be based on research findings regarding their effectiveness in the space environment.

27.6 Postflight Support

Space voyagers have to deal with the return to the Earth environment and the adjustment to a more usual routine. Personal and marital problems that the crew member tried to ignore during the mission may become more evident upon return. Moreover,

family problems may become exacerbated while the voyager is in space, particularly as the length of the mission increases. Therefore, access to and encouragement in making use of individual and family counseling is necessary, either directly through the particular space agency, or else through referral sources outside of the agency. The latter may be more comfortable for astronauts and their families who desire privacy and to alleviate concerns that the disclosure of problems could jeopardize assignment to future missions.

27.7 Concluding Remarks

The success of most human space missions and the numerous examples of ambitious tasks that have been accomplished are generally taken as evidence of the ability of most astronauts to perform and cope in space, both as individuals and as teams. Despite this, psychological and interpersonal challenges associated with long-duration missions must not be ignored. A positive development in the history of space exploration is that greater attention is being paid to the importance of psychological factors in mission success by all national agencies. Along with this recognition, psychological countermeasures have been more accepted as part of mission planning. Such countermeasures have the potential not only to help astronauts maintain performance and well-being, but also mitigate the potential for health problems due to immunosuppression and other physiological alterations partially induced by stress. Evidence-based research is required to support the use of countermeasures, as well as the most effective methods of crew selection, training, and in-flight and postflight psychological support. For this purpose, high fidelity ground-based models of spaceflight are of great value to supplement the limited flight studies in this area.

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Physical Countermeasures to Stress

28

Vera Abeln and Stefan Schneider

28.1 Introduction

Since the first Apollo missions, exercise in space has been an important field of research (Halberg et al. 1970; Rummel et al. 1973, 1975). For scientists it has always been a major interest how fundamental physiological systems as the cardiovascular and the musculoskeletal system are affected by missing gravity; how they adapt, how they degrade. A number of exercise ideas and concepts have evolved since that time, to minimize both, the effects of microgravity on degenerative processes as well as the amount of time that needs to be spent on exercise.

Besides its impact on the cardiovascular and musculoskeletal system, physical activity is also known to affect brain cortical function and to have a positive impact on psychophysiological parameters (Schneider et al. 2009b), on host defense and health. Especially endurance training is regarded as a pertinent compensatory activity for different kinds of stress (Hollmann and Strüder 2000). So, in addition to the positive effects of exercise on the peripheral physiological system (Convertino 1996, 2002), it is assumed that adequate, individually specified exercise programs will help to maintain possible neurocognitive decrements of long-term confinement (Lipnicki and Gunga 2009) as well as counteract the effects of confinement on the mental health status in space (Palinkas and Suedfeld 2008) and result in an improvement of general well-being and mood.

Although in general positive effects of exercise on musculoskeletal and neurocognitive processes predominate, for further space missions it needs to be taken into consideration that specific problems might evolve out of different exercise programs. Besides the fact that individual exercise preferences need to be considered

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A Holistic Approach to Exercise: The Balance of Activity/Inactivity (ora et labora)

An important number of papers in the last decades have addressed the benefit of exercise on physiological and psychological well-being. Nevertheless, apart from some very detailed and complex physiological mechanisms, there are some anthropological mechanisms that might explain the power of exercise.

Looking back physical habits of the human being underwent a dramatic change within the last 30 years. Throughout the evolution of mankind, until the late 1970s when the industrial age ended, working life was widely categorized by physical work (from hunters to pitmen). Unsurprisingly recreational exercise and sports was not very common before the 1970s as the physical work load during a working day did not allow for much further energy expenditure. In contrast, today working life is widely characterized by nonphysical work. Accordingly exercise has developed as a key variable to stay physically and mentally fit. This has been acknowledged by a recent initiative of the American Society of Sports Medicine (ACSM) "Exercise is medicine".

With regard to the underlying metabolic and hormonal changes caused by exercise, it can be assumed that a balance of activity and inactivity is important for a general well-being. This has been identified already by the regula benedicti, which sums up in the traditional "ora et labora," pray and work. Whereas "labora" was physical work, the prayer was characterized by physical recovery. So one might postulate that exercise itself is not important for a mental well-being but it is the adequate alteration between inactivity and activity. Therefore it might be regarded as counterproductive and stress evoking when people, living in space are forced to exercise beside a physical stressful working day (see concrete recommendations).

(Schneider et al. 2009a), exercise, if not adequately applied, might provoke additional stress and, therefore exercise recommendations for further space missions need to be carefully defined.

28.2 Exercise in Space: Ideas and Concepts to Prevent and to Counteract Physical Stress

With the end of the industrial age and the beginning of the information age in the late 1970s, physicians realized that regular exercise and an active lifestyle is a helpful tool to substitute missing physical workload and to prevent civilization diseases as diabetes, cardio-vascular disease, and an increasing obesity among all age groups. Since then a tremendous amount of research activities and publications has validated the positive effects of exercise on the cardiovascular system,

the musculoskeletal system, on the immune system, as well as the metabolic system. With the beginning of manned space flight in the late 1960s it was realized that the loss of physical work load caused by weightlessness results in a decrease of muscle mass, bone density/stability, and cardiovascular changes. A new area of exercise science was born in this time, and research has very well documented the positive effects of exercise on the cardiovascular and the musculoskeletal system. This research has helped scientists to understand fundamental principles of musculoskeletal and cardiovascular deconditioning and to develop countermeasures, which are nowadays used, for example, in the rehabilitation of patients suffering from the negative effects of immobilization, for example, after surgery. However, their interaction with other organ systems (e.g., immune system) especially in space are warranted in future investigations.

In the first decades mainly standardized gym equipment such as treadmill and bike ergometry have been used to exercise in space. However, this exercise takes up to 2 hours off of the precious work time every day. Therefore in the recent years more efficient, new devices have been developed and evaluated (e.g., Fly Wheel, Short Arm Human Centrifuge (SAHC), vibration training) in order to increase efficiency by maximizing the output and minimizing the time spend for exercising.

Today an "exercise in space" program is guided by three fundamental principles, which all are aiming to counteract the physiological stress of weightlessness. Exercise in space aims

1. To counteract a loss of muscle mass/muscle force in order (a) to protect work capabilities and (b) to accelerate postflight recovery.
2. To counteract cardiovascular deconditioning as to prevent postflight orthostatic intolerance.
3. To counteract osteoporotic deconditioning to facilitate postflight recovery (Fig. 28.1).

28.2.1 Exercise, Weightlessness, and the Muscle

Despite a well-designed and intensive (up to 2 h/day) exercise program, it still seems impossible to counteract the loss of muscle mass and muscle strength during long term-space flights. In a recent study, Gopalakrishnan et al. (2010), report a decrease of 15% muscle mass and approximately 20% of isokinetic strength in the plantar flexors of four subjects although they were regular exercisers. This deconditioning is more emphasized in the early phases of spaceflights, which exponentially decreases in the following weeks. So far it is not clear whether this deconditioning levels out and shows no further decline. Recreation follows a similar pattern with rapid improvements in the early phases postflight. It needs to be mentioned that muscular deconditioning mainly affects the locomotor system. Muscle mass and strength for the upper extremities show no major changes and also the strength losses in the ankle dorsiflexor group was negligible, probably due to the regular use of foot loops, which are used to align and move the body against the resistance of inertial forces.

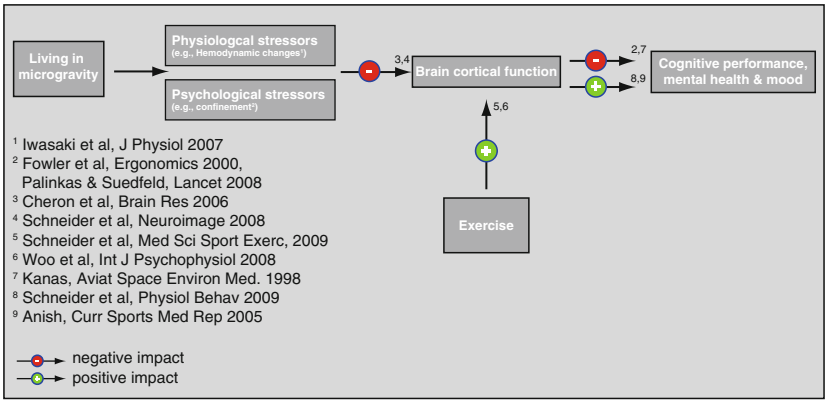

Fig. 28.1 Living in microgravity is known to result in a multitude of stressors resulting from physiological changes (e.g., hemodynamic changes in the brain) in combination with psychological effects caused by the confined situation (e.g., workload, limited nutritional supply, isolation) exist while living in space. This has a negative impact on brain cortical activity, cognitive performance, mental health, and mood. In contrast, exercise is known to have a positive impact on brain cortical function, performance, mental health, and mood. It is therefore proposed that, individually specified exercise programs might help to counteract the negative impacts of microgravity on brain function

28.2.2 Exercise, Weightlessness, and the Cardiovascular System

As recent study by Verheyden et al. (2010) has shown that under weightless conditions cardiovascular parameters (heart rate and mean arterial pressure) are similar to those of being in a supine position on earth. It is concluded that the cardiovascular system is chronically relaxed in space due to an increase of venous return and cardiac output (Norsk et al. 2006; Verheyden et al. 2010). Instead of deconditioning they name it neural adaptations to changed gravity conditions and in doing so it is indirectly marked that, despite postflight orthostatic problems, these adaptations have no negative impact on in-flight health.

Unfortunately, exercise itself is not able to provide a blood pressure gradient from head to feet in space, which would be helpful to counteract orthostatic intolerances postflight. As a result, a combination of low body negative pressure (LBNP) and exercise was proposed, and showed promising results. The data suggest that treadmill exercise using a graded lower body compression suit, and 100 mmHg lower body negative pressure provides equivalent or greater physiologic stress than similar upright exercise on Earth (Hargens 1994).

28.2.3 Exercise, Weightlessness, and the Skeletal System

Exposure caused by routine exercise stimulates bone formation and prevents bone loss, whereas unload causes a loss in bone density. Although, as compared to the muscle system, the decline in bone markers during spaceflight and recovery

postflight occurs much slower (recovery may require 1–3 years (LeBlanc et al. 2000)) several similarities between muscle and bone exist. Those similarities include a very large inter- and intra-subject variability, a distinct localization with significant losses in the lower extremities and no significant changes measured in the upper body (e.g., arms) (LeBlanc et al. 2000). Most importantly it is worth to note that a loss of bone density AND muscle mass/strength cannot be completely prevented by exercise.

In summary, it can be concluded that exercise in space is simply used to facilitate postflight recovery processes on the organ systems as described above. Although some authors account for the necessity to exercise in order to maintain work productivity in space or to promote a physical healthy lifestyle, the main tenor today is to counteract musculoskeletal and cardiovascular deconditioning in order to prevent postflight physical stress. As unfortunately no tool, no exercise routine, is able to completely prevent physical deconditioning so far, a number of new ideas are currently implemented in space exercise research, such as artificial gravity produced by a short arm human centrifuge (SAHC) or vibration augmented resistive exercise. The main attempt is to reduce precious work time spent on exercise by increasing exercise effectiveness. Although in general one might support those approaches, we need to take into consideration that during long-term trips to Mars and beyond, time limitations seem to be negligible and a packed schedule will probably be replaced by boredom and the need to entertain oneself. Moreover, these attempts so far are guided by physiological definitions and rarely take into account that there is a need to exercise beyond counteracting physical stress and facilitating postflight recovery processes.

28.3 The Effects of Exercise on Brain Cortical Function, Cognitive Performance Mood and (Mental) Health

While regular physical activity is primarily recommended for its beneficial effects on cardiorespiratory health and fitness, habitual exercisers often cite the positive effect of exercise and physical activity on mood and general well-being. There is a growing body of evidence to support the positive psychological effects of exercise, and this is acknowledged in a recent report of the US Surgeon General on Physical Activity and Health. Yeung (1996) reviewed 81 studies that investigated the influence of a single bout of exercise on mood and mental state. The vast majority (85%) of these studies found an improvement in mood and mental state with exercise, and this benefit seems dependent on the duration and intensity of the exercise (Lind et al. 2005; Ekkekakis and Lind 2006). The mechanisms underlying the link between exercise and acute changes in mood are not clear, but it is likely that changes in the concentration of different neurotransmitters (Buckworth and Dishman 2002; Hollmann and Strüder 2003) and alterations in central neural activity (Hall et al. 2007) play a role.

Also the effects of physical exercise on cognitive performance have been studied throughout the last 30 years. While research concentrating on simple motor performance tasks after exercise provided inconsistent results (for review see Tomporowski

2003) recent studies, using complex decision-making tasks have provided clear support for an improvement of cognitive performance during and after exercise (Brisswalter et al. 2002; Anish 2005; Lo Bue-Estes et al. 2008; Mierau et al. 2008). One of the main contributing factors for enhancement of cognitive performance seems to be the increase in the level of arousal related to physical exercise, which seems to affect central nerval processes in those brain areas responsible for emotional and cognitive processing (see also Chap. 5).

In spite of a general agreement that exercise improves mood and cognitive performance, there is an ongoing debate about dose–response effects of exercise (Ekkekakis and Petruzzello 1999; Ekkekakis et al. 2000). Recent evidence suggests that the transition from aerobic to anaerobic exercise metabolism seems to have an impact on mood (Hall et al. 2002). Furthermore, there is a consensus that changes in mood should be monitored on an individual level as experiments on a group level might blur important variations (Ekkekakis and Petruzzello 1999; Schneider et al. 2009b; Brümmer et al. 2011).

In the last 5 years, there has been an increased interest in detecting the underlying neurophysiological processes of these behavioral findings. However, research is still in its early stages as standardized brain imaging methods as positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) are limited in an exercise setting. An overview of the applicability of different imaging techniques during and after exercise was presented in a recent edition of the Methods journal (2008,Vol. 45, Issue 4 - selected pages 253–342). So far we can conclude from several studies that exercise does not only affect cortical activity in regions that have been identified to control motor activity like the sensory-motor cortex or supplementary motor areas (SMA) but also areas that have recently been connected with emotional and cognitive processes, especially (pre-)frontal cortex (PFC) areas. From affective neuroscience and exercise psychobiology, it is known that the PFC plays a major role in regulating and coping with emotions such as fatigue, distress, and tension (Faw 2003). Today, two main theories exist which describe and explain the effects of exercise on PFC areas: (I) the transient hypofrontality hypothesis and (II) the dual mode theory.

28.3.1 The Transient Hypofrontality Hypothesis

Dietrich's (2006) transient hypofrontality theory assumes that exercise reduces activity within the prefrontal cortex. This idea is based on the assumption that cerebral blood-flow remains stable during exercise and that an increase in oxygen consumption in regions that are directly involved in motor control (e.g., motor cortex, sensory cortex, SMA, etc.) coercively results in a decrease in blood flow in regions that are not directly involved with exercise, like the PFC. The hypofrontality theory is supported by PET findings (Tashiro et al. 2008) showing a decreased prefrontal cortex activity during exercise. A further study conducted by Schneider et al. (2010) showed also an increase in alpha frequency which is a decrease in cortical activity immediately after exercise. Nevertheless, this theory is limited by the fact that it is not able to explain why specifically frontal brain areas are affected. It might also be

occipital or temporal areas that show a lowered activity if we just assume an on/off mode caused by the increasing metabolic demand of exercise.

28.3.2 The Dual-Mode Theory

A second conceptual idea, which is close to the hypofrontality theory, is the dual-mode-theory brought up by Ekkekakis (Ekkekakis and Lind 2006; Ekkekakis 2009). Within the framework of a dose–response relationship of exercise, it is assumed that with ongoing exercise, intensity cognitive factors, originating mainly in prefrontal cortical areas, systematically shift toward interoceptive factors such as muscular and respiratory cues. "As this happens, the multiple subcortical routes directing interoceptive information to the amygdala are believed to become the primary mode of affect induction" (Ekkekakis and Lind 2006).

Following both theories of a shift of cortical resources away from regions responsible for cognitive and emotional processing *during* exercise, one might speculate that even *after* exercise, previously "overloaded" brain regions are now downregulated due to the increased computational demand of brain regions associated with exercise. If we regard the brain as a multiprocessor unit, then exercise is able to reduce clock frequency in specific processors (because calculating capacity is needed elsewhere) and this seems to reset cognitive as well as emotional processors and might explain positive effects of exercise on emotional as well as neurocognitive performance. A less technical, more resource-orientated model would assume that, during exercise, the brain is, with increasing intensity and duration, more and more concerned about keeping the physical system running. If there are limited resources available this will prevent from dealing with emotions (you will be definitely not worry any more about the question whether your wife still loves you or not while spending the 10th minute at 90% VO_{2max}) or with cognitive processes you won't be able to worry about your company's next year's business plan at this intensity). After exercise, the tremendous workload will result in physical relaxation, which is accompanied by neurophysiological relaxation. Body and mind need to recover from an exceptional physical experience and from organizing this experience, and there is some experimental evidence to confirm this understanding: Tashiro et al. were able to demonstrate that the adjusted regional metabolic rate ratio is increased in sensorimotor and premotor but decreased in temporal and prefrontal cortex areas while running (Tashiro et al. 2008). Within two recent studies, Schneider et al. reported a decrease of cortical high frequency activity (beta-activity, desynchronized state of the brain, indicator for excitatory brain activity) in areas that are responsible for language processing (Brodmann areas 21/22) (Schneider et al. 2009c, 2010), as well as an increase in alpha activity (synchronized state of the brain, indicator for decrease of brain cortical activity) in prefrontal cortex areas (Schneider et al. 2010). Apart from that, an increase in cortical alpha frequency was reported in somatosensory areas after exercise (Schneider et al. 2009c). This indicates on a cortical level that exercise results in physical relaxation, which is definitely going along with cortical relaxation.

28.3.3 A Neurotransmitter Theory

Besides these theories that are more focused on changes in regional brain cortical activity, changes within the neurotransmitter system, especially dopamine and serotonin might be considered to be involved in the improvement of mood and cognitive performance. There is good evidence that the neurotransmitter serotonin plays an important role in the etiology of mood changes. The precursor of serotonin Tryptophan (TRP), but not serotonin (5-HT), can pass the blood–brain barrier (BBB) via a carrier system, in which TRP and large neutral amino acids (LNAA), especially the branched-chain amino acids (BCAA), compete for transport into the brain (Fernstrom and Wurtman 1971; Pardridge 1977). If TRP entry from blood across the BBB into the brain is attenuated, central TRP concentration as well as consecutive 5-HT biosynthesis is increased (Chaouloff 1989; Strüder and Weicker 2001a). Experimental depletion of TRP induces a transient lowering of mood in control subjects (Klaassen et al. 1999), and can induce an acute depressive syndrome in patients actually remitted from major depression (Delgado et al. 1990). In contrast, a sufficient amount of aerobic physical exercise >30 min is known to affect the TRP uptake across the blood–brain-barrier positively, which consecutively increases serotonin biosynthesis (Strüder and Weicker 2001a, b). In parallel, previously reported effects of exercise on cognitive performance, mood, and mental health might be correlated with a release of neurotrophic factors like brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1), vascular endothelial growth factor (VEGF), and prolactin (PRL) (Rojas Vega et al. 2006, 2008). In those studies, an increase of BDNF during physical exercise was shown and from animal studies it is well known that the release of brain BDNF triggers neurogenetic processes in different brain areas such as the hippocampus and prefrontal cortex areas (van Praag et al. 1999).

The effects of exercise on brain functions and mood can of course also be discussed in the light of other phenomena like the *runners high* as caused by opioidergic mechanisms (Boecker et al. 2008) or endocannabinoids (Butterly et al. 2010) (see also Chap. 8). Unfortunately it seems – at least from individual reports – that the *runners high* is hardly predictable nor reproducible but seems to be connected to a number of framework conditions which are hard to entitle ("sometimes it works – sometimes not"). Moreover a *runners high* seems to be connected to a personal bias to running. At least first findings indicate that a *runners high* is limited to exercise intensities and durations far beyond what is used for health aspects.

To sum up, for now we can only speculate about the underlying neurophysiological processes caused by exercise. Existing approaches to access to the brains' electrical and neurochemical (sub)systems during exercise are restricted. This includes limited access by MRI imaging techniques and the fact that the analysis of *peripheral* blood is not of much support due to the impenetrability of the blood–brain-barrier (BBB) by several of the mentioned neurotransmitters and neurotrophic factors. However, new technologies have provided modern tools to get more insight into neurophysiological changes of the brain in the course of exercise. Just recently, software-based electrotomography, in combination with recently developed active EEG

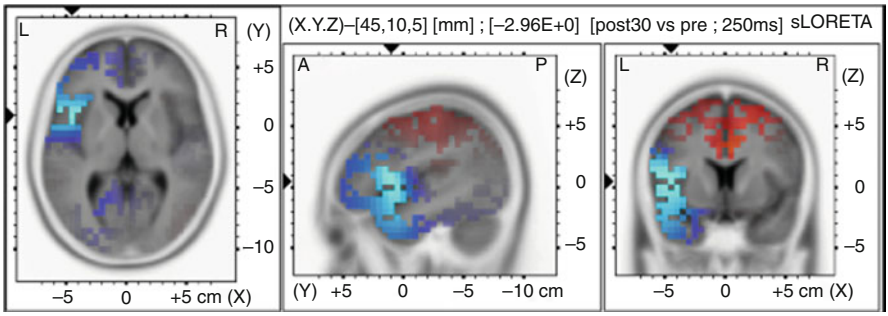

Fig. 28.2 Statistical parametric maps (SPM) of standardized low resolution brain electromagnetic tomography (*sLORETA*) differences in the EEGs high frequency activity (13–35 Hz) comparing 30 min postexercise at 80%VO_{2max} with pre-exercise ($n=12$). *Red* and *yellow* indicate increased activity and *blue* indicates decreased activity. Several studies have shown that a significant decrease of activity occurs in frontal and left temporal lobes, including Wernickes' Area, which is well known to be connected to language processing (Schneider et al. 2009c, 2010). It is concluded that a postexercise decrease in these areas might facilitate cognitive processing

systems, allows to localize exercise-induced changes in brain cortical activity, supporting a transient hypofrontality theory. In contrast to standard brain imaging techniques, electrotomography therefore allows an easy to use tool for brain imaging even under extreme conditions like exercise or in weightlessness (Fig. 28.2).

28.3.4 Exercise Immunology

As physical health plays a major role on mental well-being, this last paragraph is briefly dedicated to the effect of exercise on the immune system. Shephard has recently reported the need for a more integrative view of exercise and its positive and negative effects on the immune system functions and disease (Shephard 2010). This is likely because exercise can affect different immune responses as a function of the duration, degree, and type of the exercise. This includes several organs as well as a redistribution of immune cells (Adams et al. 2011) and direct functional control of innate and adaptive immune cells (see Chaps. 9–13) through complexly orchestrated levels of interaction including (1) direct brain region and nerval structures (e.g., the autonomous nerve systems (ANS), see Chap. 6) (2) neurohumoral (e.g., catecholamines) or hormone-like stress mediators (e.g., endocannabinoids, NPY) as well as metabolites released from either degradation of energy rich phosphates during strenuous exercise or from direct release from stressed and damaged cells (see Chap. 13) (Choukèr et al. 2005). These and other yet not fully understood interactions of mechanisms affect immune cell proliferation and function (Giraldo et al. 2009) hereby finely tuning immune functions and either resulting in higher resistance to infection or tumor growth, or inversely to higher risk of disease (Kakanis et al. 2010).

This ambiguity of immune activation and suppression became evident in a study reported after strenuous endurance exercise showing that both pro-inflammatory

and anti-inflammatory pathways are activated (Ostrowski et al. 1999). How this immune balance turns into a not favorable direction and which molecular mechanisms are involved in the immune regulation in response to exercise and the subsequent neural, hormonal, or metabolic pathway remains unclear but certainly involves expression of genes which are differentially regulated by different transcription factors, alternative splicing, gene silencing by small RNAs (miRNAs or miRs), or others (Wessner et al. 2010). Further research is warranted to find out to what extent and how physical exercise can affect critical immune function on Earth and in Space (Romeo et al. 2010).

In brief, this chapter tried to identify the positive effects of exercise on emotional and cognitive processes as well as the underlying neurophysiological processes. How exactly and to which degree this is affecting immune function and health remains to be understood. However, it is a major contribution of exercise science together with other fields, to provide a more detailed and more precise descriptions of the functionality of the (neuro-) physiological system and its implication on the behavioral system and "downstream" pathways affecting the immune homeostasis.

28.4 Concrete Recommendations: What to Do?

Exercise might be used as a suitable "global tool," not only for counteracting physiological stress but also to mitigate psychological stress during long-term space missions. Exercise might therefore be defined as one key factor for crew performance and mission success. The following paragraph will try to identify exercise recommendations in order to maximize a psychophysiological health outcome. These recommendations are based on simple principles how exercise affects psychophysiological health and a general well-being.

1. The current exercise recommendations on board of the ISS foresee a daily exercise routine of up to 2 h/day. With respect to the immense workload and the tight schedule that astronauts and cosmonauts experience while in space, it needs to be questioned whether a strict exercise schedule provokes additional stress physiologically as well as psychologically.
2. As described before, the balance of physical activity and inactivity is a major determinant for the well-being and health. Physically demanding activities (e.g., extravehicular activity, EVA) should not be followed by additional exercise routines the same or following day as they are likely to result in immune imbalance (as seen in athletes).
3. To further minimize the impact of exercise routines on the time management while in-flight, new time-saving approaches like vibration training or artificial gravity provided, for example, by a short arm human centrifuge should be broadly evaluated. This evaluation must include effects on cognitive performance, mood, and immune performance. For example, vibration training might be a time-saving method to counteract muscle loss and bone stability, but perhaps duration and not intensity of training has a major impact on neurocognitive and mental processes.
4. In general, healthy individuals will feel a need to exercise if they are physically underchallenged. For mission success, it is of utter importance not only

to physically train astronauts/cosmonauts but also to sensitize them in their predeparture training years to the effects of exercise on psychophysiological health and mental performance.

5. Besides all this, we need to keep in mind that an individual dose–response relationship as well as an exercise preference hypothesis exists. If we aim to optimize exercise, we need to aim for individual exercise prescriptions, taking into account individual habits and an individual bias rather than generalizing exercise on a physiological level.

28.5 Concluding Remarks

In the last 10 years, an increasing number of studies from exercise science were devoted to the positive effects of exercise on cognitive performance, mental health, and a general well-being. Exercise is a "global tool" that might help to facilitate the individual's psychophysiological adaptation to extreme conditions of life, to microgravity, and therefore to act positively on the crews' performance level, mission safety, and mission success.

However, when the role of exercise in space is discussed, there is a need to distinguish physiological aspects from health aspects. Exemplarily for this differential view, one might state that if research within the next years will show that artificial gravity is the ne plus ultra to prevent musculoskeletal deconditioning, but the individuals get sick every time they are centrifuged, this might have an overall negative effect on their health status. While physiological reactions to a given exercise stimulus are supposed to be widely similar, the emotional reactions hereto are definitely not. This reflects well, that the general idea of health does not equal physical health alone.

Throughout the last decades exercise has been regarded as a countermeasure to physiological deconditioning in space. Within this chapter, it is proposed to identify exercise not only as a countermeasure that is solving problems, but as a preventive tool to avoid problems inter- as well as intra-individually. While developing tools and programs to fragmentarily prevent musculoskeletal deconditioning as an organ-directed approach, we should also aim to enhance crew performance level as well as individual mental and physical health in a more holistic view. This integrated approach is needed because mission success is endangered by a multitude of stressors (social, workload, environment, confinement, and others) that can be likely avoided by regular and individually adapted exercise. The degree and the consequences of exercise, respectively, have to be assessed also in the light of their effects on other health relevant entities such as to the immune system. A good reason for exercising however was already acknowledged in the first century by Juvenal "*mens sana in corpore sano*".[1]

[1]Although a traditional translation to "a healthy mind in a healthy body" does not reflect the meaning of the context which is more: "we should pray to the gods that there should be a healthy mind in (=and) a healthy body."

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Nutritional Countermeasures for Spaceflight-Related Stress

29

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29.1 Introduction

Optimal function of the immune system is impaired in the presence of malnutrition. This led to the concept of immunonutrition which describes diets that are specifically designed to enhance immune functions and, without adequate nutrition, the immune system is clearly deprived of the components needed to generate an effective immune response (Beisel 1992; Mizock 2010). Nutrients act as antioxidants and as cofactors at the level of cytokine regulation (Cunningham-Rundles et al. 2005). Crews during spaceflight generally have lower dietary intake compared with normal conditions on ground (Lane et al. 1998). It is well known from ground research that a lack of macronutrients or selected micronutrients, like zinc, selenium, and the antioxidant vitamins can have profound effects on immune function (Chandra 1992; Chandra and Kumari 1994; Keith and Jeejeebhoy 1997). Micronutrient deficiency suppresses immune functions by affecting the innate T-cell-mediated immune response and adaptive antibody response. It leads also to a dysregulation of the balanced host response (Wintergerst et al. 2007). Disruption of nutritional balance and dietary intake of astronauts and cosmonauts during spaceflight, which is often accompanied by a stress response, might influence their immune response as the nutrition status can influence host immune responses and resistance to infection (Sonnenfeld 2002; Sonnenfeld

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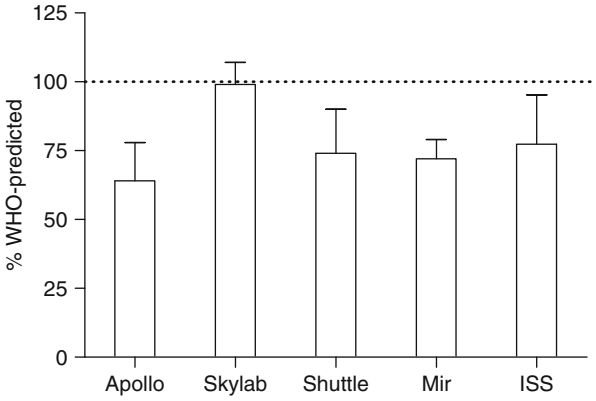

Fig. 29.1 In-flight dietary intake of crew members in different space programs. Data are expressed as percentage of energy requirements predicted by the World Health Organization (WHO) (WHO 1985). Apollo $n=33$, Skylab $n=9$, Shuttle $n=32$, Mir $n=7$, ISS $n=23$. Apollo and Skylab data are from Bourland et al. (2000). Figure is adapted from Smith and Lane (2008), with additional data from Smith et al. (2005) and Smith and Zwart (2008) (Smith et al. 2005b, Zwart et al. 2011)

and Shearer 2002). Some of the immune function alterations documented in astronauts (see Chaps. 9–12) have been reported also in states of nutrient deficiencies, and could potentially be counteracted by improving their nutrition. Detailed information on the absorption and metabolism of many micronutrients during spaceflight are mandatory before specific nutritional recommendations can be made, especially with regard to their relationship with the immune system function.

29.2 Energy Intake

During brief space flights of 9–10 days duration, energy expenditure was measured using doubly labeled water (Lane et al. 1997). This study demonstrates that energy expenditure for 13 men during short spaceflights was nearly identical to that measured in a 5-d ground-based period. Therefore, human energy requirements during space flight are – at least in short-term missions – similar to those on earth. Another study, on a similar mission with intensive exercise, actually documented increased energy expenditure during flight (Stein 1999). However, energy intake during flight is often lower than the estimated requirements for individual crew members (Fig. 29.1). Many astronauts could be considered malnourished during their mission, meaning their appetite is decreased and thereby their nutrient supply is inadequate (Bourland et al. 2000) leading to body mass loss during flight (Fig. 29.2).

A study on the Russian Mir space station assessed the urinary excretion of 8-iso-prostaglandin-F2 alpha and 8-oxo-7,8-dihydro-2 deoxyguanosine (8-OH-dG), markers for oxidative damage in six subjects during and after long duration spaceflight (90–180 d) (Stein and Leskiw 2000). Although urinary isoprostane decreased significantly during flight, 8-OH-dG excretion tended to increase. A similar observation was made

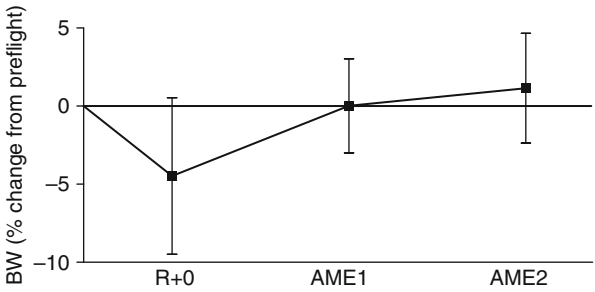

Fig. 29.2 Postflight body weight (*BW*) of Mir and ISS crew members ($n = 20$). Data are expressed as mean ± SD of the percent change from preflight body weight. *R + 0* landing day, *AME1* first annual medical exam after return from the mission, and *AME2* second exam (Smith et al. 2009)

on the Life and microgravity Science shuttle mission. The regression of the excretion rate of 8-OH-dG against the energy deficit of either Mir or Life and Microgravity Spacelab (LMS) showed that the greater the decrease in energy intake, the more extensive free radical propagation occurs because of the diminished protein-based antioxidant defense mechanisms (Stein 2002).

29.3 Protein and Amino Acids

Protein and amino acid deficiencies can have profound effects on a variety of immune system functions (Chandra et al. 1984; Guadagni and Biolo 2009). When the body is deprived of energy, protein is one of the most important limiting factors, because essential amino acids are not stored in the body. There is evidence that inactivity amplifies the catabolic response of skeletal muscle to inflammatory mediators (Biolo et al. 2006). Guadagni and Biolo (2009) concluded that optimal protein (amino acid) intake could be much greater than the minimum amount of protein required to decrease whole body protein wasting. This concept of optimal protein intake to promote non-anabolic actions of specific amino acids could also be applied to microgravity. Microgravity is a state of physical inactivity and physical inactivity is associated with a low-grade inflammatory condition, as Steffen et al. (Steffen and Musacchia 1986) demonstrated in a study of experimental bed rest in healthy volunteers. Results from this bed rest study showed that physical inactivity might also be associated with increased protein requirement.

Moreover, Stein et al. (Stein and Gaprindashvili 1994) used the ^{15}N-glycine method to measure whole body protein syntheses in-flight. The studies showed that whole body protein synthesis rates were increased by approximately 30% on the second and eighth day of spaceflight, with decreased nitrogen balance. At the same time, urinary cortisone, fibrinogen, and IL-2 levels were elevated, suggesting that the increased protein synthesis was due to stress. Stein et al. suggest that spaceflight triggers a stress response similar to injury-induced stress.

The defined spaceflight requirement for protein intake is 0.8 g/kg per day, not to exceed 35% of the total daily energy intake (Smith and Zwart 2008; Smith et al. 2009). About 2/3 of the total amount of protein is to be provided in the form of animal

protein, and 1/3 of the total should be in the form of vegetable protein. A study by Heer et al. [21] showed that, on long missions, reaching (or exceeding) nominal protein intakes is common, but that on short flights (Shuttle missions) protein intake is less than the recommended amount because of insufficient food intake (Heer et al. 2000b). An energy deficit, as seen during short-duration spaceflight (Lane et al. 1997), will also lead to loss of protein. However, varying the type of protein might attenuate protein loss by increasing postprandial net protein synthesis as shown by Antonione et al. (2008), when comparing the postprandial net protein synthesis following whey versus casein protein during head-down tilt bed rest. The rapidly digested whey protein was more efficient in counteracting the reduced postprandial net protein synthesis than the slowly digested casein (Antonione et al. 2008). Caution must always be taken in interpreting protein or amino acid supplementation studies, given the inherent energy content of protein. Experimental designs often fail to capture the effects of energy supplementation alone, and rather compare an energy restricted group to a protein supplemented group, with inherently confounded outcomes.

29.3.1 Arginine

Arginine is a necessary amino acid for normal T cell function and may become essential in catabolic states. Many studies have shown improved immune response following arginine supplementation as part of immunonutrition (Van Buren 2004; Bronte and Zanovello 2005; Joyner 2005; Popovic et al. 2007; Ralph et al. 2008). Supplementary dietary arginine has been shown to have useful effects on cellular immunity in animal studies, showing an increased thymic size, enhanced lymphocyte proliferation to mitogen and alloantigen, augmented macrophage and killer cell lysis, and increased lymphocyte interleukin-2 production and receptor activity (Kirk and Barbul 1990). Supplementation of arginine led to improved wound healing and immune responses in elderly subjects (Kirk et al. 1993). Based on these observations, it might be promising to supplement arginine during long-term missions; however, no studies have been carried out up to now testing arginine as a measure to improve immune response during space missions.

29.3.2 Glutamine

Glutamine is the most abundant free amino acid in the body. It can inhibit NF-κB activation and cytokine expression following sepsis (Singleton and Wischmeyer 2008). Beneficial effects of glutamine are, beside others, antioxidant effects, as a precursor to glutathione as well as an energy substrate for lymphocytes and neutrophils, and stimulation of nucleotide synthesis (Wischmeyer 2007, 2008). Glutamine seems to have a significant benefit on mortality, length of stay, and infectious morbidity in critical illness (Wischmeyer 2008). Positive results of glutamine supplementation have been shown in critically ill patients in whom supplemental glutamine

reduced complications and mortality rates (Novak et al. 2002). However, up to now the use of glutamine as a pharmaconutrient has not been tested in spaceflight or spaceflight analogs (i.e., bed rest).

29.4 Vitamins and Minerals

29.4.1 Vitamin D

Although Vitamin D can be derived from nutrition, the main supply of Vitamin D_3 (cholecalciferol) is obtained through photosynthesis in the skin, by exposure of skin to ultraviolet (UV) light (270–300 nm). Once in the circulation, to obtain the biologically active metabolite $1,25(OH)_2D_3$, Vitamin D_3 must first be hydroxylated in the liver into 25-hydroxyvitamin D_3 ($25(OH)D_3$) and then in the kidney by the $25(OH)D_3$-1-hydroxylase into $1,25(OH)_2D_3$, Vitamin D_3 (calcitriol). The latter step is under control of parathyroid hormone; however, in case of vitamin D deficiency/insufficiency, renal hydroxylation becomes substrate dependent, that is, dependent on the circulating 25(OH)D (Schleithoff et al. 2008). Vitamin D status can best be assessed by measuring circulating 25(OH)D concentrations (Zittermann 2010). The 2011 DRI report from the Institute of Medicine (Institute of Medicine 2011) states that 50 nmol/L is optimal for serum 25(OH)D concentration to maximize benefits for bone; however, this number does not take into consideration the potential benefits for immune function, which may require higher serum concentrations. Cut-offs for defining vitamin D deficiency are in the range of 25–30 nmol/L for deficiency, >125 nmol/L as of concern, and ≥250–500 nmol/L for intoxication (Zittermann and Gummert 2010; Institute of Medicine 2011).

Most of the astronauts in space stations like Mir or ISS do have low 25(OH)D levels due to almost absent natural UVB from sunlight and limited food sources (Rettberg et al. 1998; Smith et al. 2009). Indeed, it was reported in 2005 that decreased vitamin D status was one of the most striking nutritional changes that occurs during spaceflight (Smith et al. 2005a, b). We showed that the mean level of 25(OH)D concentration of US ISS crew members is 62 ± 14 nmol/L (Smith et al. 2009), given the initial ISS supplement provision of 400 IU per day. Serum 25(OH)D concentrations of not only the crew members on the Russian space station Mir, but also ISS astronauts have typically been about 30% lower after a 4–6 month space flights, and in several crewmembers, even decreased to levels considered clinically significant (Smith et al. 2005b). The current documented spaceflight requirement for dietary intake of vitamin D is 25 μg per day (National Aeronautics and Space Administration Johnson Space Center 2005), an increase from the original ISS recommendation of 10 μg per day (National Aeronautics and Space Administration Johnson Space Center 1996). The ISS food system provides less than half of this amount (4 μg per day on average) (Smith et al. 2009). Given this shortfall, and because astronauts in space are shielded from sunlight, considering them to be in a high-risk group seems appropriate. It is currently recommended that ISS crew members take 800 IU of vitamin D per day during long-duration spaceflight (Smith et al. 2009).

The classical function of vitamin D is to regulate calcium homeostasis and thus bone formation and resorption. However, recent publications show that vitamin D exerts also other biological activities including immunomodulation. The latter seems to be mediated via the (nuclear) vitamin D receptor (VDR) expressed in antigen-presenting cells and activated T-cells (Van Etten and Mathieu 2005). Vitamin D and the VDR are required for normal numbers of regulatory T-cells. The discovery that VDR is inducible expressed by lymphocytes following activation suggests a role for $1,25(OH)_2D_3$ in the immune system (Mathieu and Adorini 2002). Moreover, even the enzyme 25(OH)D3-1-α-hydroxylase is expressed by active macrophages, enabling to synthesis and secretion of calcitriol (Hewison et al. 2003). However, here the enzyme is mainly activated by immune signals such as interferon (IFN)-γ rather than the parathyroid hormone as the mediator in the kidney (Van Etten and Mathieu 2005). Moreover, the active Vitamin D metabolite $1,25(OH)_2D_3$ can also modulate by alternative mechanisms to increase the ability of PBMC from sensitized human donors to resist to microbes (here mycobacteria). Martineau et al. found that $1,25(OH)_2D_3$ suppressed both bacillus Calmette Guérin (BCG-) infected cell cultures and MTB likely through "nonclassical" mechanisms including the induction of antimicrobial peptides (Martineau et al. 2007a, b).

Studies during or after spaceflight have shown numerous changes in astronauts' immune status, including altered distribution of circulating leukocytes, altered production of cytokines, decreased activity of natural killer cells, decreased function of granulocytes, decreased activation of T-cells, altered levels of immunoglobulins, latent viral reactivation, altered virus specific immunity, expression of Epstein–Barr virus immediate early/late genes, and altered neuroendocrine responses (see Chap. 16). Furthermore, there is evidence that a higher vitamin D status in individuals with high serum cortisol wintering-over in the Antarctic is related to a lower probability of viral shedding in saliva (Zwart et al. 2011). Hence, the deficient vitamin D status of astronauts during space missions might impact the immune status of astronauts. Further studies are mandatory to distinguish between the effects of vitamin D deficiency and mere microgravity effects.

29.4.2 Vitamin B_6

Vitamin B_6 is essential in the biosynthesis of nucleic acid and protein by providing one-carbon units used in the production of deoxythymidylate and purines affecting DNA and mRNA synthesis. Antibodies and cytokines are built up from amino acids and therefore require vitamin B_6 as a coenzyme in their metabolism. A deficiency in vitamin B_6 impairs lymphocyte maturation and growth in human, as well as antibody production and T-cell activity (Wintergerst et al. 2007).

Weightlessness has been shown to reduce the cross-sectional area of muscle fibers and is associated with a change from type I to type II muscle fibers (Kraemer et al. 2000). Since vitamin B_6 is stored mainly in muscle tissue (Coburn et al. 1988), a decrease in muscle cross-sectional area could reduce the amount of the vitamin that is stored. Increased excretion of 4-pyridoxic acid (4-PA) during bed rest, a finding

observed in short- (Zwart et al. 2009) and long-duration bed rest studies (Coburn et al. 1995), likely reflects this loss of muscle stores of vitamin B_6.

29.4.3 Vitamin B_{12}

Vitamin B_{12} functions in many enzymatic reactions, and deficiencies result in anemia, as well as neurological disorders. Vitamin B_{12} functions as a coenzyme in two metabolic forms: adenosylcobalamin and methylcobalamin. Vitamin B_{12} works as a cofactor for three different enzymatic reactions: (1) the conversion of homocysteine to methionine, (2) the conversion of L-methylmalonyl-coenzyme A (CoA) to succinyl-CoA, and (3) the isomerization of L-leucine and β-leucine. Vitamin B_{12} deficiency may cause the accumulation of folate in the serum because of a reduction in B_{12}-dependent methyltransferase, also known as the methyl-folate trap (Shin et al. 1975). Vitamin B_{12} also functions in the synthesis of choline, which can be converted to the neurotransmitter acetylcholine.

Unlike other water-soluble vitamins, vitamin B_{12} can be stored in the body for years. It is stored predominantly in the liver, but smaller amounts can also be found in the muscles, kidneys, bones, heart, brain, and spleen. About 2–5 mg of vitamin B_{12} is stored in the body (Institute of Medicine 1998). The size of B_{12} stores remains relatively stable, partly because urinary and fecal excretion decrease in direct relationship to decreases in the body pools. The half-life of vitamin B_{12} in humans is 350–400 days.

A human study in vitamin B_{12} deficient patients evaluated the alterations of immunological indicators following administration of vitamin B_{12}. In these patients, the number of lymphocytes was significantly decreased and NK cell activity was suppressed. Supplementation with vitamin B_{12} reversed these effects indicating that it may act as a modulatory agent for cellular immunity (Tamura et al. 1999). In elderly subjects (aged 70 years) who received over 4 months in addition to the regular diet a special nutritional formula providing, among other nutrients, 120 IU vitamin E, 3.8 mg vitamin B12, and 400 mg folic acid, NK cell cytotoxic activity increased in supplemented subjects, indicating increased innate immunity in elderly people (Bunout et al. 2004). These few studies demonstrate the importance of a sufficient vitamin B12 status to maintain an adequate immune response (Maggini et al. 2007).

The current documented spaceflight requirement for dietary intake of vitamin B_6 is 1.7 mg/day (National Aeronautics and Space Administration Johnson Space Center 2005) and vitamin B_{12} is 2.4 μg/day. No data are available on vitamin B_6 or B_{12} status during or after long-duration spaceflight. Therefore, the interaction between the immune system and vitamin B_6 and B_{12} status has not been investigated up to now in space flight.

29.4.4 Sodium

Sodium is the major cation of the extracellular space and as such responsible for water and electrolyte metabolism. Sodium intake, mainly as sodium chloride, is

very high in the Western world, being 2,379 mg/day for women and 3,216 mg/day in men in Germany, which is above the recommended intake per day of 550 mg/day (Max-Rubner-Institut 2008). Sodium intake in space missions (Skylab and Shuttle missions) averaged 4,000–5,000 mg/day, and were similar to preflight values (Bourland et al. 2000). In the ISS missions, typical intakes have been in excess of 4,500 mg/day and in some cases intake as high as 7,000–10,000 mg per day have been observed (Smith et al. 2009).

High sodium intake is correlated with development of hypertension in sodium sensitive people. We have shown in spaceflight as well as in ambulatory conditions that already at a level of about 4000 mg/day sodium is retained without accompanying fluid retention (Drummer et al. 2000; Heer et al. 2000a, 2009). The hypothesis how sodium can be bound in an osmotically inactive way has been brought forward by Titze et al. (2003, 2004) and proposes that sodium can be stored on proteoglycans in interstitial sites. This uniquely bound sodium can induce a state of local hypertonicity in the skin interstitium. In a further study, they suggest that the local hypertonicity is sensed by macrophages which then activate a transcription factor (tonicity-enhanced binding protein (TonEBP) which, in turn, induces VEGF-C signaling (Machnik et al. 2009; Marvar et al. 2009). Macrophages play a key role in innate immunity and therefore further studies in microgravity should distinguish between the effects of microgravity and high sodium intake on the immune system.

29.5 Antioxidants

Endogenous antioxidants play an important role in minimizing cellular damage (Stein 2002). The antioxidant vitamins A, C, and E are cofactors in the immune response. Vitamin A deficiency impairs mucosal barriers and diminishes the function of neutrophils, macrophages, and NK cells (Stephensen 2001a). Vitamin E is a strong antioxidant that can support monocyte/macrophage-mediated responses (Park et al. 2003) and vitamin C is a regulator of redox and metabolic checkpoints controlling activation and survival of immune cells (Wintergerst et al. 2007).

Selenoproteins are an important component of the antioxidant host defense system affecting leukocyte and NK cell function (Ferencik and Ebringer 2003).

29.5.1 Vitamin A

Vitamin A plays a well-known role in immune function and protection against infections (Ross 1992; Stephensen 2001b). Vitamin A deficiency can affect host defenses directly through its essential functions in metabolism in the various immune cells (Semba 1998) or indirectly through its role in epithelial cell differentiation and host barrier function (Ross 1992; Stephensen 2001b). The considerable immune benefits, which would contribute in reducing the risk of various pathogen-mediated diseases, warrant a recommendation to supplement individuals with minimal or poor vitamin

A status. However, if there are immune benefits of providing additional vitamin A to those with sufficient status is not known (Field et al. 2002).

The current documented spaceflight requirement for vitamin A intake is 700–900 μg/day. Vitamin A content and stability in the space food supply should be determined. The role of vitamin A as an antioxidant in spaceflight has not been investigated up to now.

29.5.2 Vitamin C

Ascorbic acid (vitamin C) is an essential component of every living cell. The concentration of vitamin C is very high in leukocytes and is used rapidly during infection to prevent oxidative damage. A deficiency in vitamin C status is associated with reduced immune function (Schwager and Schulze 1998). The immune-enhancing role of vitamin C has been reviewed (Wintergerst et al. 2006). Vitamin C has been shown to stimulate the immune system by enhancing T-lymphocyte proliferation in response to infection increasing cytokine production and synthesis of immunoglobulins (Jeng et al. 1996).

Spaceflight requirement for dietary intake of vitamin C is 90 mg/day. Vitamin C status of crew members has not been investigated to date. However, in analog studies like short-duration bed rest, no significant change in vitamin C, but a trend for an increase, could be shown (Zwart et al. 2009). This might be related to dietary vitamin C intake during the study compared to the intake before the study (Zwart et al. 2009).

29.5.3 Vitamin E

Vitamin E and selenium have synergistic functions in tissues to reduce damage to lipid membranes by the formation of reactive oxygen species (ROS) during infections. The ability of vitamin E to scavenge lipid soluble-free radicals is dependent to some extent on the status of two other antioxidant compounds, vitamin C and glutathione, which are involved in reducing oxidized vitamin E back to a reusable (i.e., able to be oxidized) form (Carmeliet et al. 2001). Additionally, vitamin E may improve T-cell function by decreasing macrophage PGE2 production by modulating the amino acid cascade initiated by lipoxygenase and/or cyclooxygenase (Carmeliet et al. 2001; Chapkin et al. 2007). Furthermore, vitamin E influences lymphocyte maturation, possibly by stabilizing membranes and allowing enhanced binding of antigen-presenting cells to immature T cells via increased expression of intercellular adhesion molecule- 1.

After ISS crew members had spent 4–6 months in space, their plasma γ-tocopherol was 50% less than preflight levels (Smith et al. 2005b). No change in α-tocopherol occurred in these subjects.

Although no striking changes occurred in plasma vitamin E concentrations, the space flight menus provide only about 60% of the documented requirement for

vitamin E (Smith et al. 2009). Antioxidant properties of vitamin E may help to counteract the free-radical damage caused by high-linear energy transfer radiation in space. Pretreatment with antioxidants may help decrease radiation damage during mission (Pence and Yang 2000), and it may be necessary to provide enough vitamin E so that astronauts' blood levels of the vitamin is higher during spaceflight than on Earth. However, knowledge gaps weaken the evidence for use of vitamin E as a countermeasure.

29.5.4 Copper

Copper is an important mineral, and has wide-ranging functions, including many that are considered vital for spaceflight (Borchers et al. 2002; Crucian et al. 2008; Tipton et al. 1996; Taylor et al. 1997; Levine and Greenleaf 1998). This might have direct or indirect (when alterations are induced by psychological stress or radiation stress) implications for nutrition and nutritional status as possible causes or effects on immune system function (Smith et al. 2009). Nonetheless, to date, little/no information is available about copper metabolism during space flight.

29.5.5 Zinc

In addition to its many essential functions in growth and development, zinc is essential for the function of cells of the immune system (Kumari and Chandra 1993). It has an important role in promotion of wound healing and in maintenance of intestinal integrity. A deficiency of zinc is also associated with reduced concentrations of IGF 1 and reduced rates of protein synthesis. Therefore, zinc deficiency could be especially detrimental during immobility. However, zinc status of astronauts, as assessed by mean serum zinc and urinary zinc excretion (admittedly, not the best markers of zinc status), did not change after long-duration spaceflight (Smith et al. 2009). There is no knowledge available on the use of zinc supplementation as a countermeasure in spaceflight.

29.5.6 Polyphenols (Resveratrol, Quercetin, Curcumin, Catechins)

Naturally occurring polyphenols like resveratrol, quercetin, curcumin, catechins have shown antioxidant and anti-inflammatory effects (Chung et al. 2010). These effects seem to be modulated via different pathways such as NF-κB- and mitogen-activated protein kinase-dependent pathways, as well as preventing the generation of reactive oxygen species by binding iron (Perron and Brumaghim 2009). Additionally, polyphenols seem to activate sirtuin 1 (SIRT1) directly or indirectly

and thereby are beneficial – besides others – for regulation of oxidative stress, inflammation, and autoimmunity. Accumulating evidence has shown that polyphenols such as resveratrol, curcumin, catechins, and quercetins, have a regulatory role in immune function in vitro and in vivo (Gao et al. 2003; Song et al. 2003; Devine et al. 2007; Sharma et al. 2007; Singh et al. 2007; Shim et al. 2008; Park et al. 2009). Therefore, they might also have beneficial effects in prevention of immune dysfunction during long-term missions, particularly because body iron stores are higher during spaceflight. However, the role of polyphenols in SIRT1-mediated or iron-related regulation in immune function remains still to be studied. No results are obtained during spaceflight up to date. Therefore, it is very obvious that further studies are also warranted to examine polyphenols as potential dietary measures to counteract immune dysfunction in microgravity.

29.5.7 Iron

Iron is a critical micronutrient involved in many cellular processes. Functions include oxygen binding, electron transport, and serving as a catalyst for literally hundreds of enzymes. Iron deficiency can have irreversible consequences, but excess iron can be toxic through the formation of oxygen free radicals. Iron overload can increase cardiovascular disease risk and cancer risk, impair immune function, and contribute to eye diseases such as cataracts and age-related macular degeneration (Crichton 2009; Loh et al. 2009). Thus, maintenance of iron homeostasis is extremely important for human health.

During spaceflight, it is well established that iron homeostasis is altered (Smith et al. 2009). There is a decreased red blood cell mass, increased serum ferritin, decreased transferrin receptors, and increased serum iron – all of which document increased iron storage during spaceflight. Furthermore, the space food system provides almost three times the recommended intake (Smith et al. 2009), at a time when dietary iron recommendations for the general population being lowered. This can in turn bring iron into an even more ambiguous "role," as it is not only necessary for host survival, but microorganisms require iron acquisition from the environment for survival as well. Generally, during an infection, iron in the body is made less available for invading microorganisms by increasing iron uptake into cells or by increasing protein-bound iron. Cells of the innate immune system have genes, which regulate proteins that can modulate iron homeostasis at the cellular and systemic level in order to restrict iron availability to invading microorganisms. One such protein is hepcidin, a key regulator of iron homeostasis and critical factor in the anemia of inflammation (Ganz 2006). Hepcidin has been shown to be endogenously expressed by innate immune cells – macrophages and neutrophils, and it plays a role in making iron less available by increasing intracellular iron sequestration and decreasing circulating iron concentrations and it is influenced by cytokines IL-6 and IL-1 (Nemeth et al. 2004; Lee et al. 2005).

Further research is warranted to determine what role, if any, increased iron storage during spaceflight plays on changes in immune function and microbial growth and virulence.

29.6 Polyunsaturated Fatty Acids (PUFAS)

Omega-3 fatty acids are long-chain, polyunsaturated fatty acids (PUFA). Extensive documentation exists showing that omega-3 fatty acids provide protection to the general population for the cardiovascular and immune systems (Yaqoob and Calder 2003; Fernandes et al. 2008; Kang and Weylandt 2008), and even protection from oxidative damage and radiation-related risks (Turner et al. 2002; Vanamala et al. 2008), all of which are concerns for space travelers.

The mechanism of action of omega-3 fatty acids on these systems is likely related to multiple pathways, but there is evidence that the nuclear transcription factor, NF-κB, is affected differently by omega-3 or omega-6 fatty acids (Camandola et al. 1996). This transcription factor affects transcription of genes involved in cell cycle regulation and inflammatory processes. Not only is NF-κB activated by arachidonic acid and specifically by prostaglandin E2, but Camandola and colleagues have also found that eicosapentaenoic acid (EPA) inhibits NF-κB activation (Camandola et al. 1996).

Cytokines such as tumor necrosis factor α (TNFα) can activate NF-κB, and subsequently stimulate other processes such as muscle protein loss through upregulation of mRNA and expression of various ubiquitin-proteasome protein subunits (Li and Reid 2000). Not surprisingly, conditions associated with a chronic elevation of TNFα are also commonly associated with muscle atrophy. We have reported elevated NF-κB after short-duration spaceflight (Zwart et al. 2010). The effects of omega-3 fatty acids on inflammatory cytokines, and specifically TNFα, are well documented on the ground (Kang and Weylandt 2008; Kim et al. 2008; Magee et al. 2008; Zwart et al. 2010), but warrant further studies during spaceflight.

29.7 Conclusion

Immunonutrition has emerged as a field critical for improving outcomes in a wide range of patients, particularly in under/malnourished individuals. Since astronauts in space are generally not optimally nourished, dietary formulas tailored to the astronaut's needs may be beneficial for immune system function. Furthermore, the environmental stress of spaceflight can lead to changes in immune response as well as the individual needs of the respective astronaut. These and other factors are required to support optimal astronaut health during long-duration missions.

However, it is important to be aware that "one size does not fit all." This implies that the immune nutrient profile that is appropriate for one astronaut or one condition may be of minimal benefit for another one and could be potentially harmful in other settings. Making evidence-based decisions in choosing the optimal diet or formula will minimize adverse effects. In order to reach that level of individualized immunonutrition for astronauts, further research is needed to assess whether immune function is benefited by individually tailored immunonutrient formulas.

The use of basic clinical pharmacology, molecular biology, and clinical research principles in the study of nutritional therapy during spaceflight and analog studies will lead to answers on how to administer the right nutrients, in the right amounts, at the right time during astronauts' space missions.

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Pharmacological Countermeasures to Spaceflight-Induced Alterations of the Immune System

30

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30.1 Introduction

Opportunities for microbes to establish infections are enhanced under spaceflight conditions because space travel stimulates their growth (Chap. 15) and has a negative impact on immune functions. Indeed, it has been shown that spaceflight affects lymphoid organs (Gridley et al. 2003; Baqai et al. 2009) and induces variations in peripheral blood leukocyte subsets (Chap. 9). Neutrophil, monocyte, and NK cell functions are affected by spaceflight (Chaps. 10–12). The activation of T lymphocytes is also severely depressed under low gravity conditions (Cogoli et al. 1984) because interleukin-2 (IL-2) and IL-2receptor gene expression are modified, the delivery of the costimulatory signal to activate the B7/CD28 pathway and the protein kinase A (PKA) signaling pathway, which is a key early regulator in T cell activation, are hindered. Furthermore, a TH2 cytokine shift is associated with spaceflight. If this TH2 shift persists during long missions, it could represent a significant clinical risk for TH2-related autoimmune diseases, allergies, hypersensitivities, and disease susceptibility related to diminished cell-mediated immunity. Studies on plasma antibody levels did not reveal significant changes after short spaceflights (Rykova et al. 2008), but contradictory results were reported after long missions. Indeed, several studies (Konstantinova et al. 1993; Bascove et al. 2008, 2009; Guéguinou et al. 2009, 2010) reported increased levels of immunoglobulin while Rykova et al. (2008) reported normal amounts of antibodies after prolonged space missions. Lastly, a differential sensitivity of cellular and humoral immunity to spaceflight conditions seems to exist because it was shown that the cellular, but not the humoral, systems are affected by short periods of flight.

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Taken together, these data demonstrate that spaceflight-induced modifications of the immune system could have an immediate impact on mission objectives. The development of efficient countermeasures to combat the deleterious effects of spaceflight on the immune system is therefore an area that should be considered more thoroughly before we undertake prolonged space voyages. Furthermore, the observations presented above are also found in the elderly (Cancro et al. 2009) and people subjected to chronic or acute stress (see Chap. 4). Indeed, the age-associated decline in immune function, which is known as immunosenescence, is characterized by a large dysfunction in innate and adaptive immune system responses (for review see Weiskopf et al. 2009). Chronic stress reduces B and T lymphocyte responses and lowers antibody production (Glaser et al. 2000). Acute stress induces the reactivation of latent viruses, decreases NK cell activity, increases interleukin-6 (IL-6) secretion, and increases neutrophil numbers in peripheral blood (Glaser and Kiecolt-Glaser 2005). Finding countermeasures to spaceflight-associated immune alterations are therefore also of interest to counter immunosenescence and the effects of stress-inducing situations on Earth.

30.2 Effect of Combined Antioxidant Treatment

Increased oxidative stress, which is harmful for cells and can induce many disorders, has been observed after radiation exposure and is associated with spaceflight (Stein and Leskiw 2000; Wan et al. 2005). Indeed, lipopolysaccharide (LPS)-activated splenocytes from mice that flew on the space shuttle mission STS-118 produced more IL-6 and interleukin-10 (IL-10) and less tumor necrosis factor (TNF)-α than control mice (Baqai et al. 2009). The same study showed that many of the genes responsible for scavenging reactive oxygen species (ROS) were upregulated after the flight, suggesting that cells attempted to scavenge ROS produced during spaceflight. An increase in the superoxide response by murine polymorphonuclear neutrophils was also reported even after short periods of microgravity (Fleming et al. 1991). Furthermore, it was shown that the urinary concentration of 8-hydroxy-2′-deoxyguanosine, a marker of oxidative damage to DNA, was higher and that red blood cell superoxide dismutase, an antioxidant enzyme that functions as a superoxide radical scavenger, was lower in astronauts after long-duration spaceflight (Smith and Zwart 2008). Consequently, research was undertaken to determine if antioxidants could protect organisms from radiation-induced oxidative stress. Two studies showed that a mixture of L-selenomethionine (SeM), vitamin C, vitamin E succinate, alpha-lipoic acid, and *N*-acetyl cysteine improved the survival of mice after exposure to protons or to a potentially lethal dose of X-rays (Wambi et al. 2008, 2009) (Table 30.1). Pretreatment of mice with this mixture of antioxidants resulted in significantly higher total white blood cell and neutrophil counts in the peripheral blood and increased bone marrow cell counts after irradiation. Moreover, antioxidants increased Bcl-2 (B cell lymphoma-2, proteins regulating anti-apoptotic mechanisms) and decreased Bax (Bcl-associated X protein promoting apoptosis), caspase 9, and TGF (transforming growth factor)-β1 mRNA expression in the bone marrow after X-ray irradiation (Wambi et al. 2008). In mice or rats exposed to high-energy

particles radiation, D- or L-SeM or a combination of selected antioxidant agents, which included SeM, could also prevent the decrease in total antioxidants by regulating the expression of genes involved in the repair of radiation-induced DNA damage (Kennedy et al. 2004, 2007). These data indicate that antioxidants, alone or in combination, are promising countermeasures for protection against adverse biological effects from space radiation.

30.3 Nucleotides

Nucleotides are beneficial for health because they positively influence lipid metabolism, immunity, and tissue growth, development, and repair (Gil 2002). Rapidly proliferating tissues, such as those of the immune system, are not able to fulfill the

Table 30.1 Effect of countermeasures on immune parameters

Countermeasure	Experiment performed	Results	References
Antioxidants	Irradiated mice + antioxidants	– Antioxidants prevented the decrease of the antioxidant status of animals exposed to protons or high-energy particles	Kennedy et al. 2007
	Irradiated mice (X-rays) + antioxidants	– ↑ survival – ↑ white blood cells and neutrophils in blood – ↑ bone marrow cell counts – ↑ Bcl-2 mRNA in bone marrow – ↓ Bax, caspase 9 & TGF-β1 mRNA in bone marrow	Wambi et al. 2008
Nucleotides	*In vitro* Mouse splenocytes cultured under simulated microgravity conditions and stimulated with PHA + nucleotides	– Nucleoside-nucleotide mixture and uridine restored splenocyte proliferation – ↑ IL-1β, IL-2 & IFN-γ with the nucleoside-nucleotide mixture	Hales et al. 2002
	In vivo Hindlimb-unloaded mice + nucleotides	– RNA and uracil restored popliteal lymph node proliferation, PHA-induced proliferation of splenocytes, IL-2 & IFN-γ production	Kulkarni et al. 2002, 2005
	In vitro Mouse splenocytes cultured under simulated microgravity conditions and stimulated with PHA + nucleotides	– PHA-induced proliferation of splenocytes restored by uridine and nucleoside-nucleotide mixture	Kulkarni et al. 2002, 2005
	In vivo Hindlimb-unloaded mice + nucleotides	– ↑ proliferation – ↑ IL-2 & IFN-γ – ↓ corticosterone plasma level	Yamauchi et al. 2002

(continued)

Table 30.1 (continued)

Countermeasure	Experiment performed	Results	References
AHCC	Hindlimb-unloaded mice infected with *K. pneumoniae* + AHCC	– ↓ mortality – ↑ time to death and ability to clear bacteria – ↑ anti-*K. pneumoniae* IgG levels	Aviles et al. 2003
	Normally housed mice + AHCC	– ↑ spleen cell proliferation induced by Con-A or LPS – ↑ IL-2 & IFN-γ after Con-A stimulation – ↑ IL-4, IL-6 & IL-10 after LPS stimulation – ↑ nitric oxide production in peritoneal cells	Aviles et al. 2004
	Hindlimb-unloaded mice + AHCC	– No effect on splenocyte proliferation induced by Con-A or LPS – ↑ IL-2 & IFN-γ after Con-A stimulation – ↑ nitric oxide production in peritoneal cells – Restored peritoneal cell function	Aviles et al. 2004
DHEA	*In vitro* KLH-primed mouse splenocytes stimulated with KLH + DHEA	*TH2 favored* – ↑ IL-4 – ↓ IFN-γ	Du et al. 2001
	In vitro Mouse splenocytes stimulated with Con-A and LPS + DHEA	– ↓ IL-1, IL-2 & IFN-γ – ↑ IL-10 – IL-4, IL-6 & TNF-α not affected	Powel and Sonnenfeld 2006
	In vivo Retrovirus infected mice + DHEA	*TH1 favored* – ↑ IL-2 & IFN-γ – ↓ IL-6 & TNF-α	Araghi-Niknam et al. 1997
	In vivo Old female mice + DHEA	– ↑ IL-2 & IFN-γ – ↓ IL-6 & IL-10	Inserra et al. 1998

Arrows indicate up and down modulations

needs of cell nucleotides exclusively by de novo synthesis and consequently use the salvage pathway that recovers nucleotides from the blood and diet. Nucleotides modulate the immune system (Nagafuchi et al. 1997; Holen et al. 2006). They influence lymphocyte maturation, activation, and proliferation. Likewise, they affect lymphocyte subset populations in the blood and are involved in enhancing macrophage phagocytosis and delayed hypersensitivity as well as allograft and tumor responses. In addition, they contribute to the immunoglobulin response (Navarro et al. 1996; Nagafuchi et al. 1997; Maldonado et al. 2001), which has a positive effect on clearing infection. The molecular mechanisms by which

nucleotides modulate the immune system are largely unknown. Nucleotides may influence protein biosynthesis as well as signal membrane transduction mediated by the interaction of exogenous nucleosides and their receptors. They may also contribute to modulating the expression of a number of genes, including those involved in the immune system.

Because nutrient absorption and metabolism appear to be altered under spaceflight conditions (see Chap. 29), several studies have analyzed the effects of an exogenous source of nucleotides on immune function using ground-based models of microgravity. Hales et al. (2002) and Kulkarni et al. (2002, 2005) have shown that the decreased splenocyte proliferation in response to phytohemagglutinin (PHA) under simulated microgravity can be restored by a nucleoside-nucleotide mixture and uridine but not by inosine. This observation indicates that pyrimidines are more effective for immunoprotection of the hosts (Table 30.1). In vitro studies also revealed that cultured splenocytes secreted more IL-1β, IL-2, and interferon (INF)-γ in the presence of a nucleoside-nucleotide mixture. In addition, Kulkarni et al. (2002, 2005) performed in vivo studies that demonstrated that popliteal lymph node proliferation, PHA-induced splenocyte proliferation, and IL-2 and IFN-γ production, which are significantly suppressed in hindlimb-unloaded mice (a ground-based model of choice for simulating spaceflight conditions on Earth (Morey-Holton and Globus 2002)), are restored by RNA and uracil. Similarly, Yamauchi et al. (2002) showed that in hindlimb-unloaded mice, nucleotides significantly increased in vivo lymph node proliferation and ex vivo lymphoproliferation response to alloantigen and mitogens, respectively, and IL-2 and IFN-γ production. Moreover, a lower plasma corticosterone level was observed in hindlimb-unloaded mice with RNA and uracil-supplemented diet. Thus, nucleotides and especially uracil/uridine possess immunoprotective effects. These molecules are therefore potential countermeasures for the observed immune dysfunction associated with space travel.

30.4 AHCC

Another interesting compound is the active hexose-correlated compound (AHCC). AHCC is an extract prepared from cocultured mycelia of several species of *Basidiomycete* mushrooms that contains 40% of polysaccharides (β-glucan and acetylated α-glucan which are known to have immune-stimulating effects), amino acids, and minerals. Despite the fact that it is not yet an approved drug, AHCC is the second most popular complementary and alternative medicine used by cancer patients in Japan. It is available to the general public without a prescription. Its legal status is that of a functional food. AHCC may help in the treatment of cancer. Indeed, a cohort study showed a significantly longer no recurrence period and an increased overall survival rate in 113 postoperative liver cancer patients taking AHCC (Matsui et al. 2002). Another study showed that AHCC significantly enhanced cisplatin-induced antitumor effect (Hirose et al. 2007). Several studies have shown that this product has also a positive effect on human and rodent immune systems, including the enhancement of host resistance to influenza and West Nile viruses, the prevention of

thymic apoptosis induced by dexamethasone, the increase of natural killer cell activity, and the induction of IL-12 production (Burikhanov et al. 2000; Matsui et al. 2002; Yagita et al. 2002; Nogusa et al. 2009; Wang et al. 2009). Consequently, it was tested on hindlimb-unloaded mice that present decreased resistance to bacterial infections (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) (Belay et al. 2002; Aviles et al. 2003). Indeed, hindlimb unloaded mice showed significantly increased mortality and reduced mean time to death, increased levels of corticosterone, reduced ability to clear bacteria from their organs, and delayed production of anti-*P. aeruginosa* IgG antibodies, by comparison with controls. Aviles et al. (2003) showed that the administration of AHCC for one week before suspension and throughout the 10-day suspension period yielded significant beneficial effects for hindlimb-unloaded mice infected with *K. pneumoniae*, including decreased mortality, increased time to death, and increased ability to clear bacteria (Table 30.1). Furthermore, mice receiving AHCC independent of the type of treatment (hindlimb-unloaded or normally caged) had higher anti-*K. pneumoniae* IgG antibody levels. The same team later demonstrated that AHCC significantly enhanced the function of the immune system in normally housed mice but only enhanced the TH1 response in mice under hindlimb-unloading conditions (Aviles et al. 2004) (Table 30.1). Interestingly, TH1 cytokine production has been shown to be depressed after short- and long-duration missions on the International Space Station (Crucian et al. 2008). Indeed, both groups of astronauts had a low IFN-γ to IL-10 secretion ratio on the day of landing after activation of peripheral blood T cells with anti-CD3 and anti-CD28 antibodies. This observation was confirmed by another study performed on PHA-stimulated splenocytes from mice flown on STS-108, which revealed that both IL-2 and IFN-γ were significantly lower after the flight (Gridley et al. 2003) indicating that a shift toward the TH2 subset is associated with spaceflight. AHCC also restored peritoneal cell functions that are suppressed by hindlimb-unloading and increased nitric oxide production in peritoneal cells isolated from hindlimb-unloaded mice. Other studies showed that AHCC enhanced resistance to infection. In a mouse model of surgical wound infection, mice receiving AHCC were better able to clear bacteria from their systems than control animals (Aviles et al. 2006). AHCC also increased immune function that resulted in a lower bacterial load in a murine model of intramuscular infection (Aviles et al. 2008). In conclusion, AHCC appears to be an efficient immunoenhancer that restores innate immunity, which is greatly affected by hindlimb-unloading, and consequently represents another countermeasure with great potential that warrants further investigation.

30.5 DHEA

Dehydroepiandrosterone (DHEA) is one of the major circulating adrenal cortical hormones in humans and many other warm-blooded animals. This hormone is secreted by the adrenal cortex in response to stress (Kroboth et al. 1999). In the plasma, DHEA is predominantly present as DHEA-S that generates DHEA after cleavage of the sulfate group. For many years, the physiological significance of

DHEA remained elusive. However, many studies have now shown that DHEA has significant immune modulatory functions, exhibiting both immune stimulatory and anti-glucocorticoid effects (for review see Hazeldine et al. 2010). DHEA-S increases superoxide generation in primed human neutrophils in a dose-dependent fashion, thereby impacting a key bactericidal mechanism (Radford et al. 2010). In murine models, exogenous DHEA counteracts stress-induced glucocorticoid immunosuppression and increases the resistance of mice to viral and bacterial infections (Ben et al. 1999; Zhang et al. 1999). In murine model systems of aging, DHEA appears to reverse the immunological defects seen as a consequence of aging. In particular, DHEA increases the ability of old mice to resist experimental viral and bacterial disease (Daynes et al. 1993; Kalimi and Regelson 1990; Straub et al. 1998). DHEA administration also restores immune function after thermal and trauma-hemorrhage injury and reduces mortality rates from septic challenge (Knoferl et al. 2003). In addition, DHEA provides protection against several diseases, including diabetes, oncological disorders, autoimmune disease, and chronic inflammatory illness (Kalimi and Regelson 1990). DHEA appears to be a potent regulator of cytokine production supporting the idea that this molecule acts on T cells, which is the lynch pin of the adaptive immune response. However, conflicting results on cytokine production in the presence of DHEA have been reported (see Table 30.1). In vitro studies (Du et al. 2001; Powell and Sonnenfeld 2006) showed that DHEA may be an important factor for increasing TH2 cytokine synthesis, which encourage vigorous antibody production and are commonly associated with antibody responses important for resisting infection, and decreasing TH1 and proinflammatory cytokine production. However, DHEA has shown an opposite effect in vivo in which a TH2 downregulation (or TH1 upregulation) associated with DHEA administration has been found in old or retrovirus-infected mice (Inserra et al. 1998; Zhang et al. 1999; Araghi-Niknam et al. 1997). These discrepancies may reflect differences in assays used to determine DHEA effects on cytokine production or differences in animals used. Additionally, whereas in vitro DHEA is protected from biomodifications, in vivo DHEA administration could lead to rapid clearance from the blood and conversion to other steroids in peripheral tissue, which can affect T cells differently from DHEA. Despite these contradictory data, DHEA seems to be an interesting countermeasure to fight the effects of spaceflight-associated stress on the immune system.

30.6 Conclusion

The combination of antioxidants and the pharmacologic, immune-directed action of nucleotides, AHCC and DHEA show various degrees of efficiency to restore immune system alterations. Some of these molecules are able to restore one part of the immune response such as AHCC, which mainly restores innate immunity, while others, like antioxidants, have a more general action on the organism. Searching for efficient countermeasures is a promising area of research that deserves more investigation to counter or restore alterations of the immune system in Space and on Earth.

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Part VI

Perspectives for Manned Space Exploration

Platforms for Stress and Immune Research in Preparation of Long-Duration Space Exploration Missions

31

Oliver Angerer

31.1 Introduction

Future human space exploration missions will be challenging endeavors on many different levels. When considering possible immunological changes and their implications for such missions, it is helpful to understand the characteristics that exploration endeavors entail, and that are likely to affect the functions of the immune system.

These characteristics include, for example,

- Long-term isolation and confinement (a small crew living within one limited habitat for many months or even years in the case of a mission to Mars)
- Unloading of the body in freefall (or partial gravity on another planet)
- Hypobaric hypoxic conditions (under discussion for future planetary habitats to facilitate frequent extra-habitat activities)

It appears obvious that the effects of the many challenges in spaceflight are best investigated in the real space environment. With the International Space Station, orbiting in Low Earth Orbit since more than a decade, an excellent permanently manned laboratory is available. However, due to issues like limited resources for up-/download and crew time, operational constraints, limited sample sizes, etc., it is beneficial to also utilize terrestrial analogs for preparatory research. Analogs also allow differentiating the effects of different spaceflight factors, which can be helpful when making projections for new mission scenarios. Some examples of useful analogs are described in the following sections. While helpful results may also be collected using cell, tissue, or animal models, the descriptions here will focus on analogs where humans can be the test subjects.

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31.2 Bed Rest and Dry Immersion

Bed rest studies are a well established and frequently used model for many of the physiological effects related to the unloading of the human body from the pull of gravity in spaceflight. Healthy volunteers are confined to bed in a −6° head down tilt position for periods ranging from a few days to months, depending on the research objectives. During the study period the test subjects will not leave the supine position. Any activity, from eating, reading to personal hygiene or exercise will be performed lying (Fig. 31.1). The resulting muscle atrophy, bone mass loss, cardiovascular deconditioning, and other changes mimic the adaptations observed in astronauts, and thus this model can be used for research into mechanisms of the changes as well as the evaluation of measures to counter these. Basically, all major space agencies are supporting bed rest studies through their own mechanisms, and some bed rest studies have even been organized independently by investigators themselves. One critical issue is however the comparability of results or in other words the standardization of the studies. In an effort to improve that aspect, an international study group under the umbrella of the International Academy of Astronautics (IAA) has been established to develop guidelines for standardized conditions in bed rest studies.

Similar to bed rest, the dry immersion model aims at reproducing unloading effects. Here, the test subjects are immersed in big water tanks, comparable to oversized bath tubs. They are enveloped in non-water-permeable blankets, thus it is a dry immersion. This approach, which is especially popular for space research in Russia, leads to an even faster deconditioning. However, practical aspects limit the duration for which such studies can be performed.

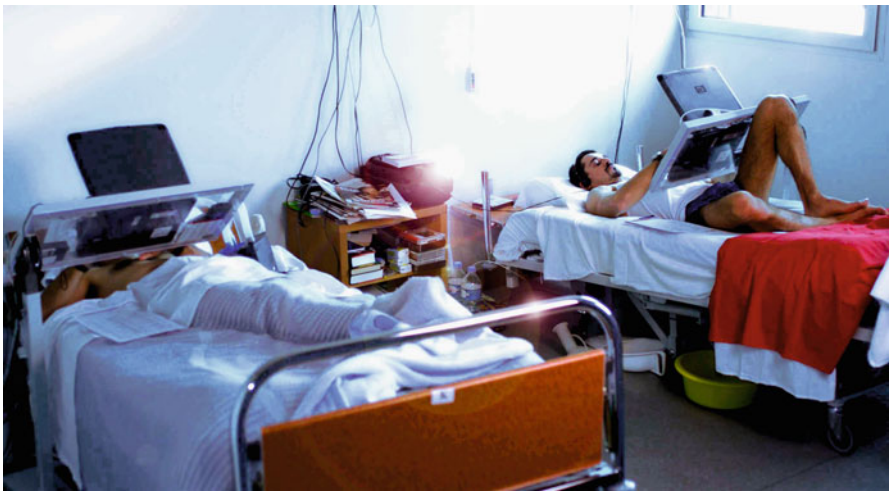

Fig. 31.1 Bedrest: Test subjects in an ESA long-duration bed rest study (©ESA)

31.3 Isolation and Confinement Studies

Isolating/confining small crews in simulation chambers has been done for different purposes in the past. Sometimes this isolation was a by-product during the testing of spacecraft systems to validate them for an upcoming space mission. At other times the aim was the testing of life support systems or elements thereof.

A further common motivation for organizing isolation studies is to investigate operations concepts and study psychological effects of long-duration isolation. During that last type of study especially, more research from other scientific fields also is implemented.

Typical crew sizes are 3–6 persons, though for special purposes or scenarios higher or lower numbers have also been used.

Many differences exist between these studies in duration, target scenario, crew selection and training procedures, and other characteristics, complicating drawing overall conclusions. Nevertheless, valuable data has been collected on a variety of topics, including the reactions of the physiological systems (including the immune system), psychological issues, the evolution of the microbial communities inside the confined space, circadian changes, as well as on some countermeasure concepts.

Hopefully in the future this approach will be further standardized and more repetitive to enhance the results. A special challenge for the future will be the integration of interacting/interfering factors like psychology, medicine, astrobiology, and life support, for example, when a crew is isolated in autonomous mode in a habitat with an advanced, closed loop life support system.

An example of current isolation and confinement studies is the MARS500 study performed in 2010/2011. This study, conducted in a facility of the Russian Institute for BioMedical Problems (IMBP) in Moscow, aimed at the replication of a full Mars mission scenario of 520 days, with a transit phase to Mars, stay in Mars orbit including simulated landing of half of the crew, and transit back to Earth (Fig. 31.2). Six crew members (three Russian, two European, one Chinese) conducted a very extensive international experiment program.

31.4 Studies in Antarctica

Dozens of countries maintain stations on the Antarctic continent. Most of the stations are located in the coastal areas, only a few deeper on the continent itself. Many are only operated during the Antarctic summer; others are occupied all year through, which due to the harsh environmental conditions typically implies a multiple-month period during which the crew is fully isolated from resupply or evacuation. While the coastal bases are at sea level, inland stations are on higher altitudes. Crew sizes range from less than ten to more than 1,000 (like at the US base McMurdo during summer).

Accordingly, the research questions that can be addressed are diverse, ranging from studies of small autonomous crews to physiological, epidemiological investigations.

Fig. 31.2 Mars500: The isolation facility used for the Mars500 study at the Russian Institute of Biomedical Problems in Moscow (©ESA)

In the spaceflight context, factors that make some Antarctic stations very interesting analogs for exploration missions are, for example, the changed day/night cycle, partially extreme isolation and therefore the need for autonomy of the crew, the hostile environment with very limited sensory stimulation, the realistic operational situation with real dangers, which can never be simulated. Further, the effects of long-term hypobaric conditions can be tested and also controlled by implementing research protocols at inner continental bases (e.g., Concordia station at ~3200 m, see Chap. 32) and at sea level, respectively. This is "bought" through some operational constraints, and through the fact that often the "test subjects" are volunteering for experiments on top of their normal main tasks, so that the overall experimental load and the level of invasiveness is less than in dedicated simulations. Therefore, these two approaches are complementary, and in fact both are required to adequately address this research.

31.5 Underwater Habitats

Diving stations, submarines, and similar underwater habitats are another classical analog for space missions. While, like in any analog or model, limitations to their use exist, they also offer realistic challenges and natural constraints and dangers. In some cases, especially for shorter campaigns, it can be easier in these settings to adopt an operational setup that mimics those used in space missions.

One underwater habitat that has been especially used by NASA is the Aquarius laboratory off the Florida coast. In the frame of NEEMO (NASA Extreme Environment Mission Operations), regular campaigns of a couple of weeks are conducted. These missions are operationally conducted like a space mission, and normally include multiple extra habitat excursions. Aims range from aspects of astronaut training, tests of technologies and tools (e.g., for telemedicine) to tests of operational concepts and provide a very interesting opportunity for research on some physiological adaption processes under realistic operational conditions.

31.6 Parabolic Flights

If studying short-term effects of true weightlessness in human beings is of interest, then parabolic flights can be a useful platform. By flying a parabola shaped trajectory with an airplane, circa 20 s periods of free fall can be produced. For some studies it can be of additional interest that this is combined with periods of hypergravity of 1.8–2 g. Specifically, with a view to exploration, more opportunities are now becoming available to also perform "lunar" or "martian" parabolas, which reproduce 0.16 g or 0.38 g, respectively, for up to 30 s. This is useful as a general gravity-related stress model, for investigating fundamental mechanisms of acute changes, as well as for testing of technologies, procedures, etc.

31.7 International Space Station

The International Space Station (ISS) is a huge international laboratory, with major contributions from NASA, ROSKOSMOS, ESA, JAXA, and CSA, orbiting the Earth at an altitude of 350–400 km (Fig. 31.3). The station is permanently occupied since the year 2000, since 2009 with six crew members. American, Russian, European, and Japanese laboratory modules allow a variety of research, including biological and physiological. For that purpose, hardware like ECG, blood pressure instruments, sampling kits, a pulmonary function system, EEG, incubators, freezers, and much more are available as research payloads on board the ISS.

Generally life science research on the ISS is very well coordinated through a working group (International Space Life Science Working Group, ISLSWG) that encompasses all ISS partners except Russia. Regular Announcements of Opportunity are published to gather experiment proposals, and the evaluation process is common for all ISLSWG partners. This coordination and cooperation makes it possible that researchers can rely on any life science payload in orbit, and are not limited to the hardware owned by their respective agency. Thus, like the ISS itself, the life science program represents a major accomplishment in international cooperation.

Next to operational issues, which may constrain specific experiments (e.g., when crews need to sleep shift in preparation for an upcoming vehicle docking), crew time as well as up- and download mass still represent the major constraints for research. Also, the environment in Low Earth Orbit and the current operational and

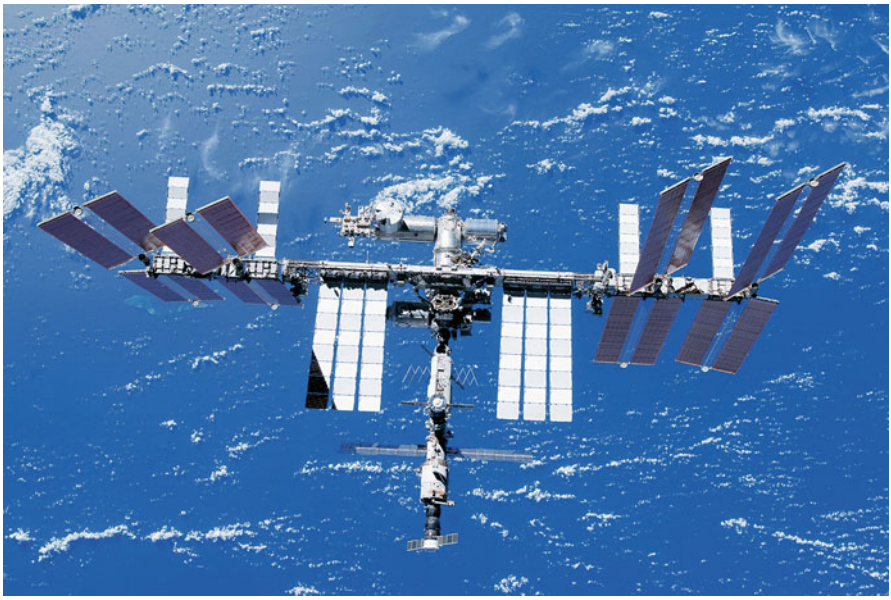

Fig. 31.3 ISS: International Space Station as seen from space shuttle Endeavour in February 2010 (©NASA)

logistical setup are different from what can be expected for exploration missions. Nevertheless, the ISS represents an excellent tool for preparatory research, as it is the only available platform in which humans are living and working in actual weightlessness for extended periods of time, within a real spacecraft.

31.8 Conclusions

This brief description of research platforms is intended to illustrate the many available opportunities for performing exploration preparation science. Crucial for success of the investigations is to select the best suitable analog for a given research question. If, for example, primarily musculoskeletal unloading is of interest and related countermeasures are evaluated, bed rest or dry immersion studies may be very suitable. If effects related to long-term confinement and isolation are a topic, then isolation simulation studies or analog environments like Antarctic stations may be useful settings. As the overall greater control and space fidelity of simulation studies with less realistic dangers are basically the opposite of not only the real situations and dangers, but also operational constraints, of analog environments, it will often be sensible to employ both settings, in order to collect complementary data.

Ultimately, most lines of investigation will require use of the unique space laboratory ISS for validation or basic data collection. However, as the cost and effort for any flight activity is high and resources like crew time are limited, it is essential that

any research project is very well prepared and optimized through the use of adequate ground-based analogs by the time the "ultimate" step to a flight experiment is made. In this way, all the platforms mentioned in this chapter are crucial and each plays an important role toward enabling our next step further out into the solar system.

Exploration Class Missions on Earth: Lessons Learnt from Life in Extreme Antarctic Isolation and Confinement

32

Alex P. Salam

32.1 Introduction

Sometime this century humans will attempt to set foot on the planet Mars. This will mark a defining moment in the history of human exploration. Never before will a journey of this magnitude or complexity have been attempted. Apart from the technical challenges, the physical and psychological threats to the crew will be without precedent. Severe derangements of numerous physiological processes will occur. The psychological state of the crew will, without a doubt, also heavily influence the mission. Prolonged separation from loved ones and our home, planet Earth, will place immense emotional strains on the crew. A life devoid of Earth's colors, sounds, and smells, will lead to sensory deprivation and monotony. Altered circadian rhythms and disturbed sleep patterns will result in cognitive and motor impairment. Endless months confined to a small habitable volume will test the patience, stability, and diplomacy of even the most highly trained astronauts. Overall, a state of constant physical and psychological stress will loom over the crew, and might indeed put their lives at risk.

I spent a year at the Concordia Antarctic research station in 2009 as a researcher in human biology and medicine for the European Space Agency, investigating the consequences of chronic stress (and chronic hypoxia) on immunity and sleep. Concordia is one of the most isolated research stations on the planet and shares many stressor characteristics with long duartion-deep space missions (LDDS). I discuss, in brief, some of the stressors present at Concordia and their consequences and similarities with LDDS missions, and potential countermeasures to disturbed behavioral health and performance.

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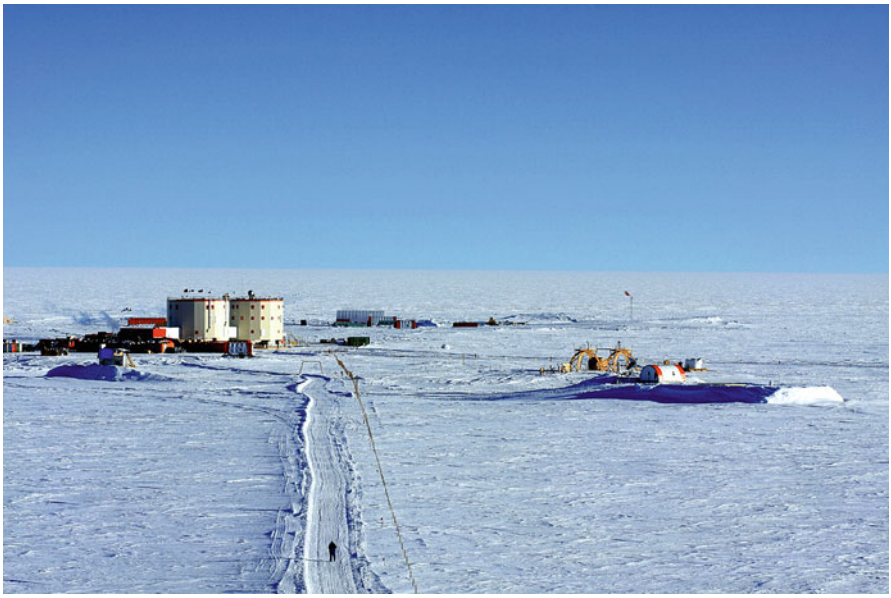

Fig. 32.1 Concordia station. The two principal towers can be seen; the 'noisy' tower which houses life support, recreation, food storage and preparation, and the mess; the 'quiet' tower which houses sleeping quarters, laboratories and medical

32.2 Concordia Station

Concordia station is located at Dome Charlie (Dome C) at an elevation of 3232 m, 1000 km inland from the Antarctic coast, on the high Antarctic plateau. Dome C is a vast barren ice desert that stretches, uninterrupted, for hundreds of kilometers in all directions. It is one of the coldest, driest, most inhospitable and inaccessible regions on the planet. This alien world was my home for 379 days. The station serves as a research platform for glaciology, astronomy, atmospheric chemistry, seismology, geomagnetism, and human biology. It is jointly operated by the French Polar Institute (IPEV) and the Italian Polar Institute (PNRA). There are only two other permanent stations on the high Antarctic plateau, the Russian Vostok station and the American South Pole station. During the summer season (mid-November to mid-February) the station is lively and there are on average 50 international scientists and technical personnel. Planes come and go, transporting people to and from the station. Snow tractors and snow ploughs arrive from the coastal station, Dumont D'Urville (distance 1200 km), bringing supplies in preparation for the winter. The outdoor temperatures are relatively mild, averaging −30ºC, and it is possible to explore the surroundings of the station before the bitter winter begins; the seismology ice cave, the astronomy platforms, the glaciology shelter, the climatology tower. The 24 hour intense sunlight encourages late "night" gatherings and the ambience is social. People are busy, occupied with station and equipment repairs, logistics, and science (Fig. 32.1).

Throughout the last week of January and the first week of February, the summer crew begin to leave and numbers dwindle until the last flight approaches. The mood and feel of the station changes significantly, becoming much more still and composed. On February 8th 2009, at 09h02 UTC, the 12 of us staying for the fifth Concordia winter-over, a mix of French, Italians, and myself, all stood side by side, waiving hesitantly as the last Douglas DC3 revved into action, kicking a mist of snow and fine ice particles towards us. A few minutes later the plane roared over our heads and rushed away into the vast expanse. The buzz of the engines dissipated, the defined form of the plane became a blur, until eventually it disappeared in an instant. This was the start of 9 months of isolation during which there was no possibility of evacuation or deliveries. The next nearest human presence was the crew of the Russian Vostok station, 560 km away. Because of its location and the environmental extremes, Concordia station is completely isolated during the winter season from mid-February to mid-November. It is simply too dangerous to attempt airplane landings or passovers, and in all likelihood technically impossible during the depths of winter. Telecommunications were limited and consisted of 2–3 satellite connections during the day for emails to be sent and received. Our personal email accounts were limited to 1 Mb per connection. There was no real time data transfer, although we did have occasional use of satellite telephone at our own expense. As individuals and as a crew, we had to be self reliant and semi-autonomous during the winter period.

On February 14th, the sun set for the first time in 3 months, marking the end of the polar summer and the start of the polar winter. It was a fleeting moment; within a few minutes the sun was back above the horizon. Over the course of several weeks however, night and day equalized and temperatures began to drop. By March, outside temperatures were averaging −45°C. By April, night had overtaken day and outside temperatures were averaging −60°C. On May 6th the sun finally disappeared beneath the horizon for three long, dark, cold months. Concordia experiences cycles of total light or total darkness because of its location deep within the polar circle. Mid-June was the coldest and darkest period of the winter, in many ways. Temperatures regularly approached −80°C (not counting wind-chill) and people were generally confined to the station for long stretches of time. On the morning of August the 11th, the sun shot a crimson flame low into the sky as it made its first, but short-lived, appearance in 3 months. By November it had returned to its 24 hour blinding fury. On November 17th, early afternoon, the exact same plane that had left us 9 months ago returned, slowly transforming from a mere glistening speckle in the desolate distance to a wonderful, thundering, colorful beast arriving just a few meters from our feet.

32.3 Stressors, Consequences and Parallels with Long Duration-Deep Space Missions

The main stressors present at Concordia station are shown in Fig. 32.2a. The list is not exhaustive. Apart from some of the miscellaneous stressors, all of the stressors listed will also occur during LDDS missions, although their intensity and the level of risk associated with them will vary. The stressors are listed under specific

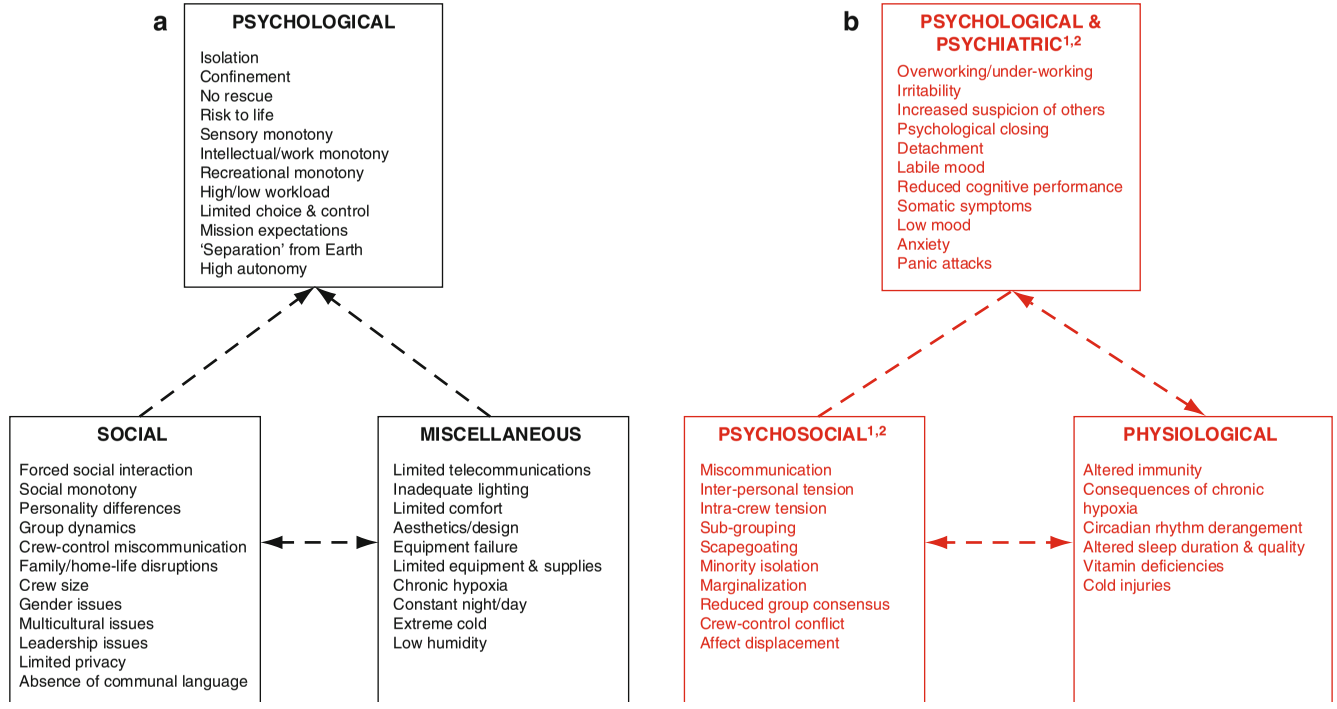

Fig. 32.2 (**a**) Stressors present during a Concordia winter-over; not listed in any particular ranking order. Many of the stressors are interrelated. Miscellaneous includes human factors, environmental factors, and habitat factors. (**b**) Consequences of the stressors; not listed in any particular ranking order. Many of the consequences are interrelated. Stressors and their sequelae feedback, in many cases, in a bi-directional manner (Palinkas and Suedfeld 2008; Lugg 2005)

categories but in reality some could be listed under more than one category, and their classification is fluid. Many of the stressors are inter-related. For example, some of the social stressors can influence the psychological stressors, and vice-versa. The intensity and numbers of stressors that an individual can tolerate is time dependent. The main consequences of the stressors are shown in Fig. 32.2b. Again, many of the sequelae are inter-related. Importantly, some of the stressors and sequelae feedback in a bi-directional manner. As a result, a single sequela experienced by a single individual can eventually have a profound impact on the group. For example, an individual feels low in mood due to their extreme isolation from society. A vicious cycle occurs as the low mood is exacerbated without access to the support of friends and family. The individual begins to withdraw from the group, and eventually forgoes some of their professional or communal duties. This can lead to the individual being made a scapegoat and marginalization from the group (see Chap. 19), which in turn amplifies the individual's mood disorder and even arouses feelings of suspicion towards others. If this individual is part of a minority group, sub-division of the group may occur. In the worst-case scenario, all these events lead to reduced group consensus, and possibly risk to the mission (Fig. 32.3).

32.3.1 Psychological Stressors

Living at Concordia is in many ways like living on a distant desolate planet. When I stepped out of the Douglas DC3 twin-engine propeller aircraft onto the ice on December 5th 2008, what struck me the most was the vast, bleak, expanse of the high Antarctic plateau. The landscape has no topography, and there is not a single drop of life or natural color in the endless ocean of listless ice that surrounds the station for as far as the eye can see. There is nothing in the environment to remind you that you are still on Earth, a planet filled to the brim with flowing water, lush vegetation, geological wonders, and wildlife. A sun that never sets and then subsequently never rises adds to this surreal sense of detachment from Earth. In addition Dome C experiences, essentially, no weather system as there are almost no clouds and there is almost no precipitation and no wind. Therefore, one feels not only very physically isolated due to the remote location of Concordia and distance from civilization, but also emotionally isolated and detached from Earth. This is compounded by the limited telecommunications and generally poor access to ample and personalized information from the outside world, including communication with friends and family. The extreme separation and isolation from close relatives and friends places a significant emotional strain on many crew members and can lead to low mood, emotional liability, or sometimes even emotional detachment. Such emotional detachment is both a positive and negative consequence of the physical and emotional isolation. It allows one to adapt to the high isolation, but when experienced to the extreme can result in emotional blunting and apathy, which in turn can result in disruptions to personal and family life, relationships that are already strained by the immense physical separation (Fig. 32.4).

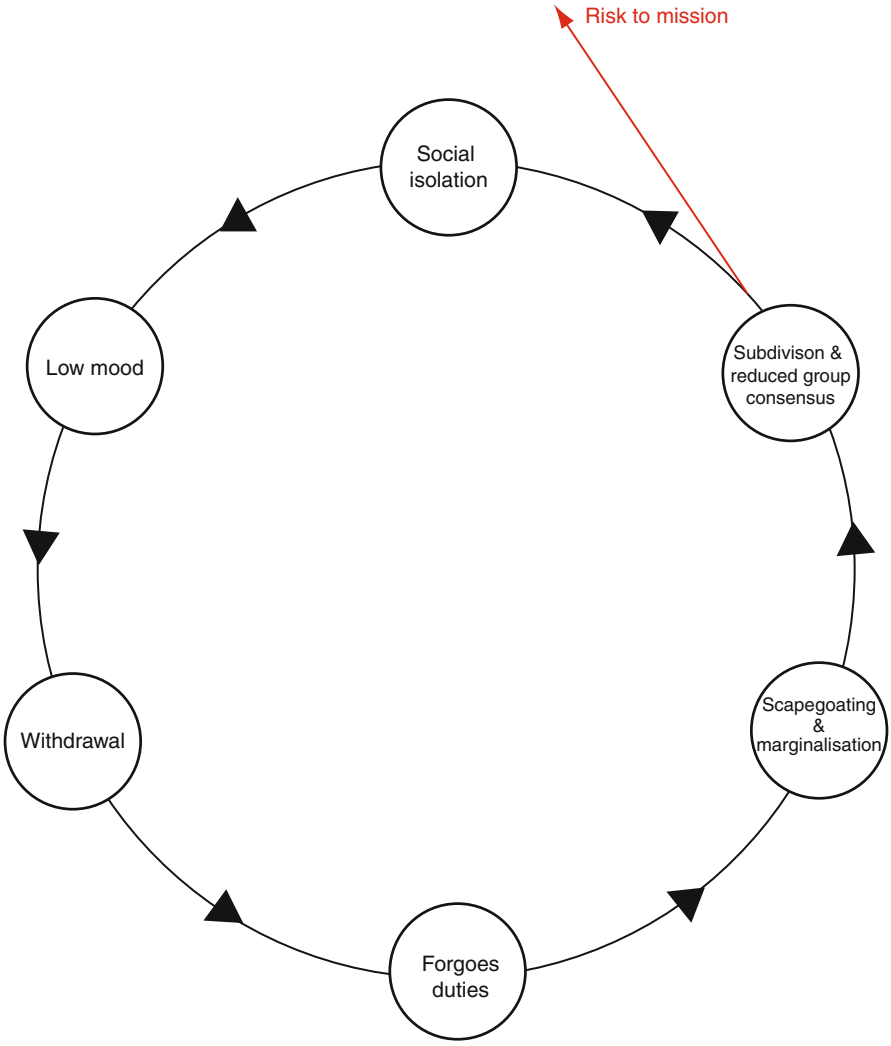

Fig. 32.3 An individual reacting negatively to a stressor can eventually have a profound influence on the group

Life at Concordia is, perhaps surprisingly, very monotonous. It is not a 'polar adventure' and in this respect is sharply different to many of the Antarctic coastal stations that have wildlife, weather patterns, dynamic landscapes, as well as shorter periods of total darkness/daylight and shorter periods of confinement. The monotony is present in several forms including sensory, intellectual/work, recreational, and social. Sensory monotony is profound. There are very few colors, sounds or smells present in the environment or the station, and those that are present are unchanging. Olfactory and gustatory senses are probably, in addition,

Fig. 32.4 Fish-eye view from the roof of Concordia station, taken during the winter, looking out onto the Dome C plateau

blunted due to the chronic hypoxia. Although food is ample, it is frozen and dried and highly dependent on the quality of preparation. The limited access to substantive and personalized information, in particular real time data transfer, amplifies the general monotony. Although friends and family could assist in retrieving information, one was wary of hassling them on a daily basis for this purpose. I managed to find a crude way round this issue by designing, with the help of a colleague back in Europe, a programme that retrieved Pubmed and Google searches through an automated email process. I would send an email to my colleague's account in Europe with an identifying title and my search request in specific syntax. At the next satellite connection, usually 8 hour later, I would receive an automated email response with the search results. I would then send the http links for the abstracts/articles I wanted at the next connection, and finally, usually 24–48 hours after my initial email request, I would receive an automated response with the abstracts/articles I desired. This was an incredibly laborious and slow process, but the only option available. I highlight this point to illustrate the extent to which we found the lack of access to scientific knowledge and information frustrating. Work was interesting for many of the scientists, but due to limited amounts of reagents and equipment, the ability to be scientifically creative or 'play' when on site was restricted. Between the biomedical experiments and my own personal goals and projects, I didn't have time for much else. My routine consisted of work, lunch, work, dinner, gym, work, socialize, bed. I, and others, followed this similar pattern almost unchanged for 279 days straight during the winter. Days all seemed to merge into one. Unexpected events and pleasures were welcome but

very rare. The major external events that occurred were the first and last sunset and sunrise, but even these events were predictable. Work served as an important adaptation method to the isolation and sensory void, but was in itself a monotonous routine for many. Again, if used to the extreme, this adaptation process had negative consequences including overworking, burnout and detachment.

Leisure activities in the station were sparse. Some of us brought recreational materials, but these were limited by transport weight and dimension restrictions. It is interesting, but perhaps obvious, to note that the motivation to pursue goals dropped soon after arrival at Concordia if these goals and pastimes were not related to Antarctica directly, to work or to pre-existing hobbies. The gym consisted of a few weight machines and a bicycle. Several months into the winter though, using the same machines and repeating the same movements was dry, and a strain on motivation. Midway through the winter I welded some steel pipes together to make a balancing beam. This helped break the repetitiveness of the gym machines and even just this small amount of variety in my exercise routine and motor functions was a huge sensory rush. Although leisure activities such as table football and a pool table were available, these were less frequently used than one might expect. This may sound surprising, but was related to choice. People didn't necessarily want to play pool or table football simply because they were available. These were not recreational activities that people had interest in prior to arriving. This lack of choice and control was noticeable in many other areas including food, work, and most importantly personal and family life. It was very difficult for those who experienced negative relationship and family events whilst at Concordia to feel as though they had any control or interventional influence over such situations.

The no abort/rescue scenario and high autonomy are subtle stressors at Concordia and not necessarily ones that are obviously felt until system failures or major medical emergencies occur. One major technical event in particular during our winter-over reminded us of how fragile we were. Around mid-winter, as endless night surrounded the station, we heard a menacing, twisting groan escape from the depths of the station in the early afternoon. The lights went out and we were plunged into darkness. The station had lost power and all systems were down. The power failure lasted over an hour whilst the technical crew worked to repair the generator systems. Human presence at Dome C is completely dependent on life support systems; electricity, heat and water principally. The summer camp, 1 km away, serves as an evacuation destination but it consists of very basic huts, simple radio communication, and one very cold, unused, generator. This event served to remind us that Concordia station is a vulnerable speck of artificially supported life in a seemingly endless expanse of uninhabited and deadly ice, and several of the crew felt a deep sense of lasting anxiety and fear as a result. Although the International Space Station (ISS) is inherently more dangerous than Concordia, the crew can evacuate and be back on Earth within 24 hours. This possibility does not exist at Concordia and we are completely at the mercy our life support systems and health during the 9-month winter. The dangers at Concordia are minimal compared to spaceflight, but it is of note that this event, in the context of no rescue possible, caused a deep sense of fear and unease in some of the crew members. It is not unreasonable to

expect that such thoughts may weigh heavily on the crew of LDDS missions as, unlike during current spaceflight, they will clearly not have the ability to abort or be rescued when millions of kilometers from Earth.

32.3.2 Psychosocial Stressors

It can be difficult to remain socially engaged and active within a group that has not been selected as a team unit. Socializing within such a small unchanging social sphere can be repetitive after many months. Forced interpersonal contact can easily reveal, sometimes at an early stage, personality incompatibilities. In a group size of twelve, in a closed and confined environment, minor disagreements can alter the mood and feel of the group significantly. Individual stress can lead to affect displacement towards other individuals, the group, and even ground control. In the case of Concordia station, the two nationality groups and two command structures can be complex and can lead to sub-grouping, which can be amplified by the absence of a communal language. The role of the leader becomes increasingly important and influential as the winter progresses, as a mediator between both individuals and sub-groups and as a figurehead. After 9 months of isolation, some individuals had seemingly irrational and suspicious negative emotions towards others and the group. As mentioned before, changes in mood of even just a single individual can theoretically, depending on the role of the individual and their interaction with the group, eventually have a profound impact. The presence of a mixed gender crew was generally a positive and calming influence, but this is clearly dependent on the personality types and the inverse can arise. Relationships often develop in isolated and monotonous environments. There is no reason to expect that this will not occur during LDDS missions when the crew is even more physically and emotionally detached from Earth. This has potentially very significant social and health implications, and is something that needs to be addressed openly when planning long duration missions.

32.3.3 Environmental Stressors

My first night's sleep at Concordia station was terrible. Dome C is one of the driest places on earth. I woke up several times with my lips cracked and stuck together, raw, almost bleeding. On my first night, at two in the morning I stirred from sleep and noticed the sun blazing fiercely through a tiny crack in the window blind as though it were midday. The light pounded my retina and suddenly I was awake and viciously alert. I couldn't get back to sleep despite the fact that after days of traveling to get to Concordia, my body and mind craved rest desperately. Within a couple of weeks I had got used to the 24 hours sunlight but I couldn't shake off the lack of humidity, and this was something that affected my sleep for several months. Cheap and rusty humidifiers, and buckets of recycled water, had little effect. By the time most of us had got used to the dryness, darkness arrived. The 3 months of total

darkness did not affect everyone equally however. Some noticed little difference in their sleep patterns and mood, whilst others required sleeping aids. Overall, people felt more fatigued, with diminished abilities to concentrate for sustained periods. During the winter, at temperatures below −60ºC outside, my eyelashes would freeze together and I would have to pry them apart to be able to see. My fingers would ping with pain as well, no matter how many gloves or hand warmers I used. Even the best polar gloves and boots offered minimal protection from the cold. Below −50ºC to −60ºC, people spent very little time outside and were confined to the station for long stretches of time. Time spent outside at such temperatures was usually a maximum of 20–30 min and mostly for technical reasons rather than recreation.

All these environmental stressors, cold, hypoxia, lack of humidity, and altered day/night cycles, result in significant physical stress. These stressors are experienced most forcefully during summer, as the change in physiological homeostasis is very acute. By the time winter begins, people have had time to adapt somewhat to the environmental stressors. Most people arrive direct from sea level and the adaptation to an altitude equivalent to 3,800 m at the equator is sudden and abrupt. Despite living at such an altitude for over a year, and many of us developing hemoglobin levels above 20 g/dL, exercise tolerance never quite matched that at sea level. It is interesting to note that during the summer, approximately 50% of the entire crew developed one or more infections, most commonly upper respiratory tract infections. This high incidence of infection was probably multifactorial and due to: a reduced ability of dry mucous membranes to act as barriers to microorganisms; the effects of hypoxia on immunity and inflammation; the consequences of sleep deprivation and fatigue on immunity; the fact that Concordia is a dynamic and high population density environment during the summer (up to 70 individuals); and the fact that people arrive on a regular basis from the outside world bringing new microorganisms with them. During the winter there were only two incidences of symptomatic infections despite the much higher levels of psychic stressors; a soft tissue hand infection that responded to antibiotics promptly, and a 24 hours febrile illness of unclear etiology that spontaneously resolved. Clearly the fact that Concordia is a closed system during the winter is a major factor with respect to this lower incidence of symptomatic infection. Wound healing however seemed, subjectively, slow. Results of the immune study CHOICE (Consequences of Hypobaric hypOxia on Immunity in the antarctic Concordia Environment) will shed light on the interaction between hypoxia, stress and immunity at Concordia (see Chap. 13). Chronic moderate hypoxia at Concordia is likely to influence not just immunity and inflammation, but many other systems and processes including, for example, cardiovascular function, cognitive abilities and muscle metabolism. Vitamins and minerals are not routinely supplemented at Concordia, and it is therefore likely that the wintering crews experience nutritional deficiencies, as well as presumably vitamin D deficiency due to the lack of sunlight exposure. Overall therefore, Concordia is not only a psychologically and socially challenging environment to live in but also a physically stressing environment.

32.4 Adaptation and Countermeasures to Stress During Long Duration-Deep Space Missions

32.4.1 Personality Characteristics and Adaptation to Stress

Many of the sequelae listed in Fig. 32.2b were experienced by one or more individuals, and the group, during the winter. Some of us successfully dealt with the issues and stressors present and were fortunate enough to remain professionally and personally motivated throughout the winter. Others unfortunately suffered psychological disturbances as described. Of these, the most serious were anxiety and panic attacks for which several individuals required benzodiazepines at one stage or another. Such psychological imbalances, perhaps paradoxically, can sometimes continue, and indeed develop de novo, upon return to civilization. The abrupt cessation of life in isolation and confinement and the subsequent re-adaptation to normality can be stressful and lengthy, and individuals are unfortunately often unprepared for this. Readjusting to social rules, routine employment and relationships can be difficult and some people experience feelings of confusion, uneasiness, disinterest and social claustrophobia. With time however, many people who have spent a winter in Antarctica feel positive effects associated with the privilege of experiencing one of the planet's most spectacularly vast and daunting environments, such as: a profound sense of accomplishment; increased personal and professional confidence; a better tolerance and adaptation to stress; a clearer vision of one's personal needs, limits and ambitions; a deeper appreciation of personal freedoms and the natural environment. Although there are many stressors present at Concordia, there is an absence of some of the stressors present in 'everyday' life, such as commuting, shopping, queues, bills, excessive choice (both restricted choice and excessive choice can, in my opinion, function as stressors), advertising and information overload, rules and regulations and so on. And although everyone feels some of the psychological and social stressors to a certain degree, some experience the absence of routine life stressors very positively. Indeed, this is probably why some people subsequently return to Antarctica for a further winter-over, even to Concordia!

Based on my observations of the crew, several personality characteristics and attitudes appeared regularly in those who remained consistently in good psychological health during the winter: detachment, lack of sentimentality, relatively unemotional yet still empathetic, stoicism, pragmatism, determination, flexibility, diplomacy, resourcefulness and creativity, and a low need for social stimulation. Admittedly, these observations were personal and possibly biased. These are not all inherent personality characteristics and some represent personality adaptations to specific stressors. In my opinion, the most critical frame of mind and approach in ensuring a successful and healthy winter-over were: having a sharply defined and almost unquestionable comprehension of one's reasons for doing a winter stay, including an obvious understanding and acceptance of the risks and sacrifices involved; the ability to keep oneself occupied and motivated, both professionally and personally, whilst ensuring time and methods for effective relaxation and recuperation; having clear and realistic personal and work goals relevant to Antarctica,

careers, or previous past-times and hobbies; a willingness to help others through personal and professional issues including the ability to appropriately re-prioritize one's own time, needs and beliefs for the benefit of other individuals and the group; resourcefulness and creativity in making the most out of the environment and limited materials available; and a certain detachment and lack of sentimentality with regards to the outside world.

32.4.2 Effective Adaptation and Countermeasures

It is unrealistic to expect humans not to suffer psychological distress when exposed to the prolonged intense and novel conditions that will occur during LDDS missions. After all, we evolved in the lush, open, vast plains of Africa, and not in a small, dark, noisy, dangerous spacecraft. That kind of environment is not, and will never be, a natural environment for humans to live in for years at a time. Expecting to identify 'super-human' astronauts and teams in advance who will successfully adapt to the stressors of LDDS missions is not realistic (see Chap. 2). Rather, helping highly trained and well-balanced individuals and teams to adapt through appropriate interventions and countermeasures seems more appropriate. The general aim of countermeasures should be to increase individual physical and psychological well-being and performance and minimize downside risks, increase group cohesion and teamwork, minimize the disruptions to families and relatives back on Earth, maximize the chances of mission success, and ensure successful re-adaptation upon return to Earth. Briefly, countermeasures can be classified along the lines of crew composition, crew training, habitability, social, recreational, psychological, sensory, work design and pharmacological (see also Chap. 26–30). Complimentary to countermeasures is the monitoring of stress via physiological (e.g., autonomic responses, biological stress parameters), psychological (e.g., questionnaires, video analysis, sociometry) and performance parameters and markers. Early identification of the consequences of stressors allows for early implementation of countermeasures. Indeed, on site analysis of physiological markers of stress (e.g., salivary amylase, neuropeptide-Y) would allow astronauts to monitor stress levels objectively themselves on a regular basis and instigate self-directed countermeasures appropriately. Some of the social and sensory countermeasures available to the crew of the ISS presently will not be present during LDDS missions. The ISS experiences stunning and ever-changing views of Earth. Visiting crews bring fresh foods, recreational materials, and private packages from friends and family. The ISS crew also has access to real-time data transfer including on demand communication with relatives and psychological counseling, and live video links. None of these stress-reducing factors will be present during LDDS missions.

Effective adaptation and countermeasures should start early, at the time of selection and training. Although all candidates for LDDS missions will undergo extensive psychological profiling and testing, and perhaps genetic characterization, the exact personality traits and attitudes needed will be extremely difficult to correctly identify in advance, for the simple reason that no one will have experienced such high levels of novel stress before. Selecting the right candidates for spaceflight is a

difficult process at the moment but will represent an even bigger challenge in LDDS missions. Testing and training of individuals and crews in advance in analogous extreme situations and environments will, without a doubt, be necessary. The Concordia winter crews are not selected as a team unit, undergo little training together prior to deployment, and do not share a specifically defined common goal. Clearly this is not ideal with regards to maximizing work and social cohesion. Crews of future LDDS missions will have to spend many years training together and, importantly, also socializing together.

There are a number of habitability factors and social and sensory countermeasures that, although limited by engineering and financial constraints, could however significantly ease the stressors during LDDS missions. For example, at Concordia station, the windows are relatively small. One large, possibly panoramic window, in the central living area, even at the expense of other windows, would have made a significant difference to the feeling of confinement. Simply being able to clearly and comfortably visualize the outside world, the Antarctic plateau, would have a calming and comforting effect. The illusion of not being physically limited by our internal environment is critical. There is psychological strength and meaning in the fact that there is a world outside the winter confines of the station. In the case of LDDS missions, earth will disappear from view relatively soon but depending on the location of windows, and orientation with regards to the sun, stars and galaxies should still be visible. In any case, the view of Earth, slowly increasing in size as the mission end approaches, will also have powerful positive psychological connotations at a time point that may prove one of the most psychologically demanding and difficult. Indeed, the view of the approaching mission destination, whether it be an asteroid, moon, or Mars, is equally psychologically important.

A minimum amount of privacy is also needed for personal recreation, sleep, hygiene, and communication. At Concordia station the communication facilities were poorly sound insulated, which meant that passersby could easily hear conversations. The ability to communicate in complete confidence with friends and family, whether real time or not, allows for the dissolution of concerns, fears and anger, which in turn helps to minimize interpersonal conflicts. Admittedly, the crew of LDDS missions will not be able to communicate in real time during most of the journey, but they will have the ability to send and receive files, and video and audio messages. The use of short messaging services and social media to communicate with friends and family will take on increasing importance the further the crew travel from Earth. Short messaging services in particular give somewhat the illusion of instant communication and would probably be very useful yet fairly simple to implement. Our private and sleeping quarters at Concordia were small and basic but thankfully solitary, which allowed us the chance to retreat when necessary. Even just a few months into the winter, I was acutely aware of the importance of being able to retreat to private space every now and then, either one's bedroom or laboratory, as a necessary adaptation aid. It is comforting to know that there is a space within your cocooned environment that belongs to your own little private world. Volume will clearly be a limiting factor during LDDS missions but it is absolutely critical that each crew member has a small but private space that can be used for personal use

and sleep, and can be defined as their own for the entire journey. The lack of humidity was a significant contributor to poor sleep at Concordia and this, and other environmental characteristics (e.g., noise, temperature, vibration and lighting), should be taken into account during the design of life support systems for LDDS habitats.

Concordia station is decorated with few colors. The water treatment unit, diesel engines, and ventilation are responsible for most of the ambient background noise. Although the most prevalent smell throughout the station was that of the water treatment unit, we were fortunate to have cooking facilities, which did thankfully provide us with pleasant aromas and a degree of sensory stimulation. Indeed the quality of food preparation was absolutely paramount. Food was the single most important pleasure we had at Concordia, and meals were one of the few occasions when the entire crew came together. The quality and variety of the food had a significant impact on the mood of individuals as well as the group dynamics. When the food was well prepared, moods and tensions improved, whereas they significantly worsened when the food was disliked. Having the opportunity to actually cook for ourselves, every now and again, and create a meal that we specifically desired was also very important. It was fun and challenging to try and make interesting and varied meals from frozen, dried, and limited ingredients. When confined and isolated for long periods of time there are very few choices allowed or available, yet even a small amount of choice can significantly alter the perception of personal freedom. In addition, in an environment where many of the sensory and social pleasures that we normally take for granted are absent, the few that are available become increasingly important and necessary.

Countermeasures to dulled senses could be relatively simple to implement during LDDS missions and might include colors, sounds, smells and possibly tactile sensations such as video projections of Earth, virtual windows, virtual reality, small plants, bottled aromas, sounds of nature, different textures, etc. Variety in motor function should also be provided, and perhaps this is best included as part of the training systems that will be necessary to keep the crew's technical skills up to date en route to their destination. It will be difficult, probably impossible, to accommodate any form of cooking during LDDS missions. It is therefore critical that adequate and personal choice be allowed for in meal selection, together with odor and flavor enhancers. There may not be enough volume to accommodate a separate relaxation area, but there should at least be a defined dining and communal area, which ideally should be separate from work areas.

Finally, the journey to and from any deep space destination will present ample opportunities for boredom and motivational insecurity to creep into the minds of the crew. Providing the crew with work and occupations during this period is vital. Above all, any work or occupation should be meaningful and relevant to the mission or the individual's needs, experience and goals. At Concordia there was a marked difference in the ability of individuals to withstand stressors between those individuals who had meaningful and plentiful work and those who did not. Due to volume and technical constraints, it will not be possible to have large volume, well-equipped and sophisticated laboratories on board LDDS missions. Any laboratory will be basic and therefore the scope for scientific creativity will be limited, much like at

Concordia. Individuals who can creatively exploit the scientific wonders of microgravity and space through their own devise with the limited equipment and materials available, or keep themselves intellectually stimulated through the use of electronic information, will be better suited to the monotonous and confined environment.

32.5 Conclusion

The stressors, consequences and countermeasures mentioned in this chapter have been discussed briefly, hoping to give an insight into some of the behavioral health issues that will affect the crews of LDDS missions. Interplanetary missions will be expensive, and physically, psychologically and technically dangerous endeavors. The stressors and consequences that have been observed during spaceflight and analogous environments, such as Concordia, will be markedly amplified during LDDS missions. Investing significant amounts of time and finance into training and countermeasures that will improve the well-being of the crew is a small fraction to pay when one considers the vast expense, expectations and risks that are at stake.

Acknowledgment Special thanks go to the DC5 winter crew, my friends and colleagues during the winter. Loredana Bessone was hugely helpful in providing support to the entire crew during the DC5 winter-over, and the crew of the ISS expedition 20 mission were also incredibly kind in sharing their experiences with the DC5 winter crew. I would also like to acknowledge the NASA Behavioral Health and Performance Group, in which my involvement has helped clarify and crystallize some of the thoughts documented in this chapter. Finally I would like to thank Dr Alexander Chouker, Dr Brian Crucian, Dr Clarence Sams and Dr Oliver Angerer for having faith in my scientific and technical abilities in isolation and confinement.

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Moon, Mars and Beyond

33

Marc Heppener

33.1 Introduction

This book deals with stress and the immune system in astronauts with a link to the importance of this research to Earth application and patients benefits However, without astronauts and space travel, the book would be devoid of practical purpose for space mission. The same is true for space travel in itself because, one might ask, what is the purpose of travel if it is not a destination? Yet, today, most spaceflights have – layman spoken – as a sole purpose to make endless turns around the Earth, without ever leaving the so-called Low Earth Orbit (LEO), which is the orbit of most inhabited spacecraft, including the International Space Station ISS, at an altitude of ca. 400 km (roughly the distance Paris-Lyon, Bonn-Berlin, London-Newcastle, Rome-Venice, or Houston-Dallas etc.). Only 24 human beings, some 40 years ago, have ever have travelled beyond.

In this chapter therefore we will look at the future perspectives of human spaceflight beyond the Earth and its very direct surroundings. Space is vast and the definition of 'direct surroundings of the Earth' can range from the Moon (380,000 km), our Solar System (distance of termination shock: ca. 25 billion km or 23 light-hours), our Galaxy (100,000 light years), our 'local group' of some 30 nearby galaxies (10 million light years). Wherever we want to go, we can make a few general statements on the nature of such a trip:

- It takes a long to very long time to reach our destination.
- The travel itself and life at the respective destination will both challenge humans by a series of known and unknown environmental stressors such as:
  - Radiation
  - Varying gravity levels
  - Closed environments
  - Toxic environments

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- The effects of these environmental factors on humans are not always well known or quantified. Yet, we need to ensure that the crew will survive the trip and is able to perform useful tasks.
- For almost all conceivable destinations, new technology will need to be developed for the travel itself, life-support, physical and mental health.

This chapter will endeavour to give a brief summary of the different aspects of extended space mission durations or even colonisation described above. The further we go – in terms of distance from Earth, and also in terms of the build-up of this chapter – the less facts are available, and the more speculative the text will become. The final paragraphs are bordering on science fiction, although still based on current research.

33.2 Consideration for Mission Planning

33.2.1 Going Places

First, the question needs to be answered what time will be required to reach our future destinations. To determine the boundary conditions of such travel, most importantly the basic orbital physics have to be taken into account.

To characterise the trip a bit better, let us examine two factors in a bit more detail. In order not to get too stressed already from reading this chapter, we restrict ourselves in this discussion first to targets within our own Solar System. The energy required to go from Earth to another planet in our Solar System, is basically determined by

- The need to leave the potential energy well of the Earth (leading to a certain so-called 'escape velocity')
- The need to reach a stable transfer orbit to the destination planet
- The need to decelerate again to reach the surface of the destination planet again with a safe velocity.
- The need to retrace our steps in reverse order, to get safely back to Earth

Each of these steps are characterised by a parameter called delta-v, describing the velocity change required to carry out orbital manoeuvres in space, and is expressed in units of km/s. Obviously, such a velocity change can only be achieved through acceleration of the vehicle, and is therefore a direct function of the efficiency of the propulsion system and the total mass of the spacecraft. It is important to realise that any increase in delta-v will have a significant impact on the mass of the space vehicle. Simply put, increasing the total velocity of the vehicle will require additional fuel, and that fuel, including its containment, will have to be accelerated also, leading to even further fuel requirements, etc. The net result is that there is an exponential relation between the ratio of useful to total vehicle mass and the effective delta-v. This relation was first derived by the Russian pioneer of spaceflight Tsiolkovsky in 1903 and carries his name. As an illustration, the take-off mass of the Apollo-16 mission was 3 million kg, whereas the mass of the lunar module was only 5000 kg; a ratio of 600.

It therefore makes sense, certainly with the currently used chemical propulsion technology, that the orbit with the most interesting characteristics, is one in which

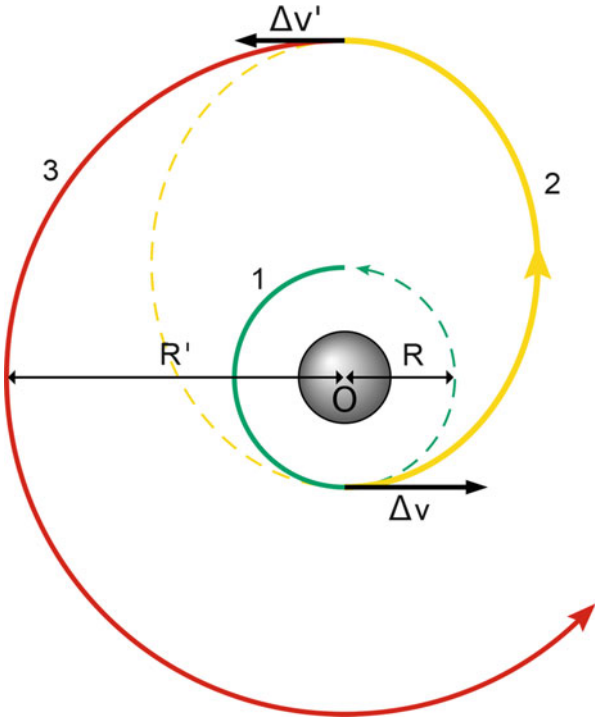

Fig. 33.1 A Hohmann transfer from a planet of origin with with orbital radius R to a destination planet with orbital radius R' (source: Wikimedia)

the space vehicle makes optimum use of the relative orbital velocities of the two planets, getting a boost from the departure planet to arrive with just about the right velocity at the destination, as depicted in Fig. 33.1. It can be shown mathematically that the transfer time of such a so-called 'Hohmann transfer orbit' is a function only of the orbital radii and the mass of the Sun. Going faster is possible, but at the costs of a significant amount of additional delta-v, whereas less energy dependent trajectories will lead immediately to much longer travel times.

Table 33.1 gives some parameters for destinations in our Solar System. From this it can be seen that, with our current technological knowledge, destinations within reach of humans are restricted to Mercury, Venus, Moon, Mars and possibly Jupiter (and in particular its moons). Anything further away is prohibitive in terms of travel times relative to a normal human lifespan.

Farther destinations would be possible if we would use different propulsion technologies, which would allow us to deviate from the Hohmann orbit. Even then, however, we have to realise that the fundamental laws of nature put limits on the velocities we can reach. Assuming that 10% of the speed of light will be a very optimistic long-term achievable goal, it will still take 15 years to reach the boundaries of our Solar system, and 42 years to travel to our nearest neighbouring star (Proxima Centauri).

The universe is immense, and it will take a really long time to reach even the closest interesting destinations. Will humans ever survive such a trip?

Table 33.1 Some specific parameters of the Solar System

	Mercury	Venus	Earth	Moon	Mars	Jupiter	Saturn	Uranus	Neptune
Distance from Sun (million km)	57.91	108.20	149.60	[0.38]	227.90	778.30	1429.40	2871.00	4504.00
Diameter (km)	4800	12104	12756	3476	6787	143800	120660	51120	49560
Density	5.44	5.25	5.52	3.34	3.93	1.30	0.69	1.28	1.64
Orbital period (days/years)	88.00	225.00	365.00	27.30	687.00	11.9 years	29.5 years	84 years	165 years
Rotation period (days)	59.00	−243.00	1.00	27.30	1.03	0.41	0.43	0.72	0.73
Gravity at equator (g)	0.38	0.90	1.00	0.16	0.38	2.64	1.14	0.92	1.15
Mean surface temperature (°C)	167.00	464.00	15.00	−153 – +107	−63.00	−108.00	−139.00	−215.00	−205.00
Surface atmospheric pressure (bar)		92	1.01		0.001	0.7	1.4	1.2	1.3
Surface composition	Basaltic	Basaltic	Basaltic/H_2O	Basaltic	Basaltic				
Atmosphere composition	Na	CO_2	N_2/O_2	–	$CO_2/N_2/Ar$	H_2/He	H_2/He	H_2/He/CH_4	H_2/He/CH_4
Number of Moons	0	0	1		2	63	60	27	17
Hohmann transfer time (days)	104.62	144.87		[3]	256.71	989.28	2196.05	5810.42	11110.96

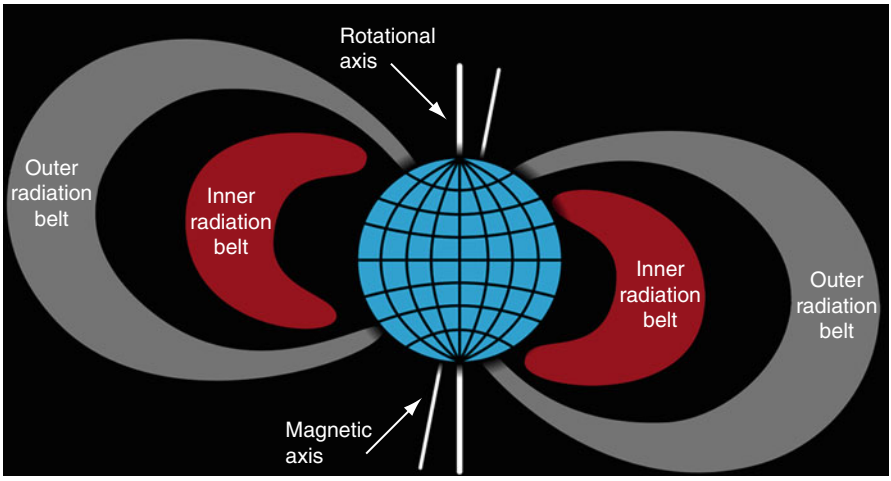

Fig. 33.2 Graphic representation of the Van Allen radiation belts generated by the Earth magnetic field and protecting the Earth from a large part of cosmic radiation (source: Wikimedia)

33.2.2 The Dragons on the Way

According to our current knowledge the most dangerous physical stress of long-distance and long-duration spaceflight is radiation. Until this very day, almost all experience in long-duration spaceflight has been restricted to the first 500 km above the Earth surface. This fact is significant because in LEO an important fraction of the harmful radiation present in space is shielded by the Earth magnetic field. In deep space, defined for the moment as beyond the Van Allen Belts (Fig. 33.2), two sources of radiation can be identified:

- Solar radiation, which mainly consists of protons. Their intensity shows a long-term variation with the Solar cycle (11 years), but can increase rapidly during periods of Solar flares and reach levels that, without proper protection, can be lethal. To illustrate the importance of this, in August 1972, just a few months after the Apollo 16 mission, a particular vehement Solar eruption took place. Would this Solar flare have happened a few weeks earlier during a Lunar EVA, the Apollo-16 crew would likely have received a lethal radiation dose (Parsons and Townsend 2000) and their perspectives of returning safely to Earth would have been bleak. Fortunately, shielding against proton radiation is relatively easy, and a relatively thin layer of water, or Lunar or Martian soil, would already provide sufficient protection. Combined with the transient nature of Solar flares, it is assumed that efficient protection strategies can be devised, although much further work is still needed.
- Galactic cosmic radiation, which is present throughout the Universe, and consists of nuclei of heavier atoms (with higher atomic number Z, such as Iron) originating from stellar nuclear fusion processes and supernova explosions. Whilst the intensity of cosmic radiation is not particularly high, shielding is

much more complicated and hence the exposure will be almost continuous. The effect of a high-Z particle impinging on a cell can also be much more dramatic than a proton event, since it creates a track of ionization of up to millimetres in length. The biological effects of this are poorly known.

Current knowledge of the radiation effects of long-duration spaceflight beyond Low Earth Orbit hence is limited. It is important to note that both the Moon and Mars have no magnetic field or dense atmosphere that could attenuate the radiation effects while on the surface. In the vicinity of Jupiter, the radiation environment is even more hostile as a result of the high concentration of cosmic radiation in its radiation belts. More knowledge is therefore required on the effects of long-term continuous exposure to cosmic radiation (Durante et al. 2010).

33.2.3 Floating Free, Falling Deep

The most visible aspect of current day spaceflight is weightlessness. In fact, this phenomenon is inherent to the free-fall of a vehicle in space being only subject to the force of gravity, such as in an orbit around a planet, or during interplanetary travel using a Hohmann transfer.

What are the effects of microgravity on biological systems? This has been an important area of scientific research in space during the past decades. With the availability of the International Space Station access to extended periods of microgravity for research purposes is now available for more than ten years. The ISS has been used to continue the study of biological effects on cells, plants, animals and humans. To increase the statistical validity, bed rest studies form a very useful analogue for studying the effects of prolonged weightlessness on the human body (see Chap. 31).

Contrary to initial hypotheses, which were based on the very small magnitude of the gravitational potential over distances of several microns, weightlessness has been demonstrated to have already an influence on the molecular processes that determine the functionality of individual cells. In the specific case of plant-roots, the mechanism of sensing gravity, transmitting this signal through a deformation of the cytoskeleton and the subsequent changes in gene expression and local changes in growth rate of the root walls, leading in the end to the curvature of the root in the direction of the gravity vector (Friml et al. 2002; Perbal et al. 2003).

For mammalian cells, our knowledge of the mechanism of gravisensing is less developed as yet, but the effects of weightlessness on signal transduction, gene expression and subsequent effects on cell division or functionality of, for example immune cells, have been well documented (see Chap. 14). Well-known effects include, for example an increased proliferation rate of several bacteria (see also Chap. 15) (Häder et al. 2005), the reduced proliferation of lymphocytes in the human body during spaceflight (Cogoli 2002), or the change in bone turnover as a result of exposure to weightlessness (LeBlanc et al. 2000; Marie et al. 2000; Scheld et al. 2001).

From the description of the effects of weightlessness at the cellular level, it is already clear that the state of the human body will change during spaceflight. While

the effects on the immune system are addressed in this book itself, further elaboration will be given on other organ systems which are affected.

- Fluid shifts and subsequent cardiovascular and hormonal adaptation processes:
  Very visible is the so-called 'puffy face' in astronauts in the first days after launch, resulting from the redistribution of fluid from the lower extremities to the head. Interestingly, adaptation processes will set in rather soon, and after a few days a new equilibrium will be reached, characterised by different hormonal levels and functioning of the cardiovascular system (cardiac output, arterial friction, etc.)
- Bone and muscle mass loss:
  Loss of calcium and overall bone-mass loss sets in almost immediately after exposure to weightlessness and at a very important rate of up to 1–2% of total bone mass per month (which is 10–15 times faster than in the most severe case of osteoporotic patients). At the same time the very long recovery time of more than a year after return to normal conditions was observed in bed rest models investigating this pathology on Earth. Similar effects arise in muscle mass (DiPrampero and Narici 2003), although here the recovery rate is faster.
- Effects on the neurovestibular and proprioceptive system:
  The equilibrium system, and in particular the semicircular canals comprise a motion-detection system that is based on the detection of fluid motion resulting from the vector-product of the gravity force and angular acceleration of the head. Clearly, without gravity vector, the output of this system is completely different and at odds with other visual or proprioceptive clues (Clarke et al. 2000). The net result can range from mild disorientation to severe space sickness in the first days of spaceflight. Interestingly, also here the human body is capable of adapting to the new environment and the effects in general disappear after a few days. In the new configuration, visual inputs have an even higher importance than under Earth gravity, and many studies take use of this effect to look into the specific mechanisms of image processing, orientation and the interaction with other proprioceptive signals. Of practical importance are tests that look into the capability of the crew in performing complex tasks such as manipulating equipment or carrying out docking or landing procedures (Viguier et al. 2001).
- Lung ventilation and related processes:
  Detailed studies have been performed on lung ventilation in weightlessness by monitoring the composition of exhaled air with or without premixed gaseous additions. The results have led to a change in the understanding of the process of gas exchange in the lungs, in particular by invalidating the assumed effect of gravity on the ventilation/perfusion ratio in the lungs (Verbanck et al. 1996). Further studies look into the potential increased risk of lung inflammation due to inhalation of floating particles, or exposure to reduced pressure environment such as in space suits during extra vehicular activities (EVA).

As can be seen from the above, the effects of weightlessness on the human body at first sight seem impressive and a potential major showstopper for human spaceflight in general. The most remarkable results of these studies is however the apparent capability of the body's organ systems to adapt to the new stressful environmental

conditions. After a few weeks of spaceflight, in general, obvious adverse effects seem to be compensated and humans are able to survive well in space for prolonged periods of time. In fact, after a few weeks, the situation of weightlessness has almost become as natural to body and mind as the normal Earth environment and new methods of orientation and locomotion (resembling dolphin-like swimming) are adopted. Humans have survived in space for extensive periods of time by now, and records include:

- Longest continuous duration in space, men: Valery Poliakov, 438 days, 1994/5.
- Longest continuous duration in space, women: Sunita Williams, 195 days, 2007.
- Longest total duration in space Sergei Krikalov, 803 days, 1991–2005.
- Longest total duration of EVA activity, Anatolyi Solovyov, 78 h 1988–1998.

In conclusion, it can be stated that the impact of weightlessness on the human body – in spite of its effects on almost every organ system – most likely seem not to be THE most critical problem for the voyage to Mars. While the number of subjects investigated during and after long duration mission are little, some long-term effects of microgravity are well documented and partly also well understood. This allows to identify suitable countermeasures to mitigate the consequences of life under the condition of weightlessness. Studies are looking into the effectiveness of various countermeasures, particularly related to drugs (Rittweger et al. 2005), nutrition or food supplements (see also Chaps. 26, 28 and 29) (Vermeer et al. 1998; Stein et al. 2003; Heer et al. 2004), exercise machines such as treadmills, resistive exercise machines (Alkner and Tesch 2004), centrifugation (Iwasaki et al. 2001), affecting different organ systems. For example, recent results indicate that bone-mass loss may be correlated with an increased sodium intake by astronauts (Frings-Meuthen et al. 2010). If true, possible countermeasures may be easy to implement, simply by adding other taste-enhancing substances to the astronauts' food than salt. It is also demonstrated in some recent bed rest studies that short-duration exposure to enhanced gravity levels in a centrifuge may counteract the effects of weightlessness on the human body (Clément et al. 2004).

A different aspect in this context is how the crew cope with sudden changes in gravity level, such as occur not only directly after launch, but also directly after landing on another planetary surface. As can be seen at any return of astronauts after a long stay in microgravity, the adaptation to Earth's gravity is stressful, cumbersome and takes roughly the same time as the adaptation to microgravity at the beginning of the flight. Some of the already mentioned countermeasure strategies may be also effective to counteract this effect. However, certainly for the immediate availability of astronauts to carry out critical tasks further research is required.

Finally, once on a planetary surface, the body will be exposed to longer times of non-zero gravity different from Earth's gravity. Here very little research is available. As long as the gravity level is between zero and one no major effects are expected. This is in a way evidenced by the limited experience of the Apollo astronauts on the Moon, and from incidental experiments aboard parabolic flights. However, prolonged exposure to higher gravity levels has never been tested in a serious way, to the knowledge of the author. Certainly an upper limit must exist for the gravity level

that a human body can sustain in a prolonged way. Astronaut training includes testing in a long-arm centrifuge operating at up to ~6 g, but the duration of this does not exceed more than several minutes. In any case, it is unlikely that an astronaut can do useful physical activities at permanent g-levels exceeding ~2 g.

33.2.4 Stressed, Bored and Lonely

Psychological factors may be the ones that will finally determine whether humans will be able to sustain very long-duration space trips. For example, a mission to Mars and back following a Hohmann transfer orbit will take some 520 days, of which roughly 1 month will be spent on the Martian surface and the rest in transit. At its largest distance, the crew will be some 360 million kilometres from home, more than 1000 times further than the Apollo Lunar astronauts. From this distance, the Earth will only be a faint dot in the sky that cannot be distinguished among the stars without aid, and communication delays may run up to 20 min one way, that is almost ¾ of an hour before a reply can be expected. In some configurations, even the Sun will be in the line of sight between Mars and Earth and visual contact and communications are virtually non-existent. Naturally, for further destinations the numbers are correspondingly higher.

During all this time, the crew may experience various forms of physical and emotional stressors. These include not only increased noise levels, limited privacy and contact with family or friends, prolonged confinement, small crew-size, increased expectations as to performance and significant risks of equipment failure or fatal mishaps, but also long periods of boredom. Prolonged exposure to such situations may affect individual psychological health, interpersonal relations in a small group, including effects related to different cultural backgrounds and language problems, leadership and teamwork, problems between the crew ('us') and ground-control ('them'), monotony, loneliness, etc. (see Chap. 19, 27 and 32). Of particular importance are the possibility for the crew to maintain not only their mental health, but also crew coherence and cognitive capabilities. Finally, as argued in other chapters and elsewhere, physical and mental health cannot be seen as separated items but as different and interacting aspects of overall crew health and performance.

To study these aspects further, long-term isolation studies are carried out. The European Space Agency ESA is performing studies in ground-based analogue environments. Important research is, for example carried out in the French-Italian Antarctic station Concordia (see also Chaps. 31 and 33). Due to its very isolated location (more than 1000 km from the nearest coastline) and small crew size of 12–16, the conditions in this base are quite comparable to those on a Martian mission. Nine months of the year the base cannot be reached by any vehicle and the crew is hence totally isolated. Several medical and psychological studies are carried out here (see also Chap. 13).

Another and even more ambitions study is the so-called MARS500 isolation study, organised and was carried out by the Russian IBMP institute in Moscow together the European Space Agency (ESA), respectively, between June 2010 and November 2011. The study simulated a complete Mars mission with six crew mem-

bers, enclosed for 520 days in a spacecraft-like complex. With exception of real space travel, weightlessness and radiation, all important aspects of a Mars mission, including communication delays, are simulated and a multitude of medical and psychological protocols is carried out to study the effects on the crew. It is interesting to note that the Concordia and Mars-500 studies are in a way complementary to each other. Concordia is a real research environment with real physical isolation. The situation is therefore on the one hand more realistic, but the environment is not well suited for testing all kind of protocols and simulated emergencies, because these might interfere with the operational aspects of the base. Mars-500 on the other hand has been a simulation, and the participants will to a certain extent stay aware of that. While this may influence the study, the environment is much more controlled, and for that reason the data will be easier to interpret.

33.3 Technological Leaps

The conclusion of the above sections can be summarised as: with technology that exists today, human spaceflight seems feasible to destination like Moon or Mars, but not much farther away. This chapter investigates a series of technological developments that are under consideration to secure this endeavour and to also extend these frontiers.

33.3.1 Health-Care in Space

During spaceflight a multitude of health risks exist. These include diseases related to spaceflight, but can also include very common diseases which need treatment. Whereas for example on the ISS in case of serious medical emergency, a crew member can be transported back to Earth in a matter of hours, during a mission to Mars there is no return possibility other than the nominal flight schedule. The consequences of this will have to be examined in every stage of the mission, from the crew selection (from risk screening to preventive medical treatment), crew training (proficiency in routine anamnesis and treatment should be present in at least two of the crew members), selection of medical supplies (even the number of painkiller, so to speak, will have to be determined in advance), diagnostic tool of minimal or non-invasive nature (see Chaps. 18–25) with adequate and personalised countermeasure algorithms up to the definition of a small operation theatre including the possibility of tele-assisted diagnosis and surgery (Haidegger et al. 2010).

33.3.2 Closed-Loop Life Support Systems

Space travellers are very human, “normal” people, and are not different in their need for some very basic consumables, such as water, oxygen and food. Although this sounds trivial, it is in fact something that asks for quite some attention. Table 33.2 shows the daily requirements of water, food and other supplies as cur-

Table 33.2 Daily consumption of consumables on the ISS per crewmember

Item	Mass (kg/crewmember/day)
Drinking water	1.5
Food water	0.5
Metabolic water	0.35
Hygiene water	1.0
Water for oxygen supply	1.0
Cooling water	0.85
Food	2
Crew items	1.0
Other	0.4
Total	8.6

rently known on the ISS. In this table it is assumed that all oxygen will be generated from electrolysis of water.

For a space mission of 520 days, which is the realistic travel time to Mars, this load would add up to approximately 27,000 kg for a crew of six. This mass however is totally prohibitive for current launchers. Clearly, recycling of waste back into air, water and food is mandatory to keep the total mass of a Mars mission under control. There are several approaches to this problem. One element is a wastewater recycling system based on nanofiltration and reverse osmosis that is currently being employed in the realistic environment of the Concordia base on Antarctica. Its capacity is some 40,000 L of water per year, with a recycling efficiency of 90%.

A more complete system would be based on a complete bioregenerative cycle, in which all water, air waste and food channels are combined (Mergeay et al. 1988). Such a system, developed by ESA under the name Melissa (Micro-ecological Life Support System Alternative) is depicted in Fig. 33.3. Of particular importance is the Photosynthesis Compartment 4, where the products of other compartments are used to produce higher plants for consumption. In 2009, the Melissa Pilot Plant (Fig. 33.4) was activated that will serve to provide actual data on performance.

Meanwhile, experiments on the ISS are carried out that demonstrate the feasibility of growing plants for food in space.

33.3.3 Chemical and Biological Dangers

Both in the spacecraft and on a planetary surface, astronauts may be exposed to an environment that may be hazardous for other reasons than just radiation (Horneck et al. 2006). Equipment has to be developed such that no off-gassing of toxic material occurs, and that dangerous chemicals will remain contained. A particular health risk results from the development of pathogens in a closed environment. In particular, in a situation where waste, water, air and food will be recycled to a large extent preferential growth of certain bacteria cannot be excluded. Also the possibility of genetic mutation of bacteria by radiation or other environmental factors has to be taken into account (Baatout et al. 2007).

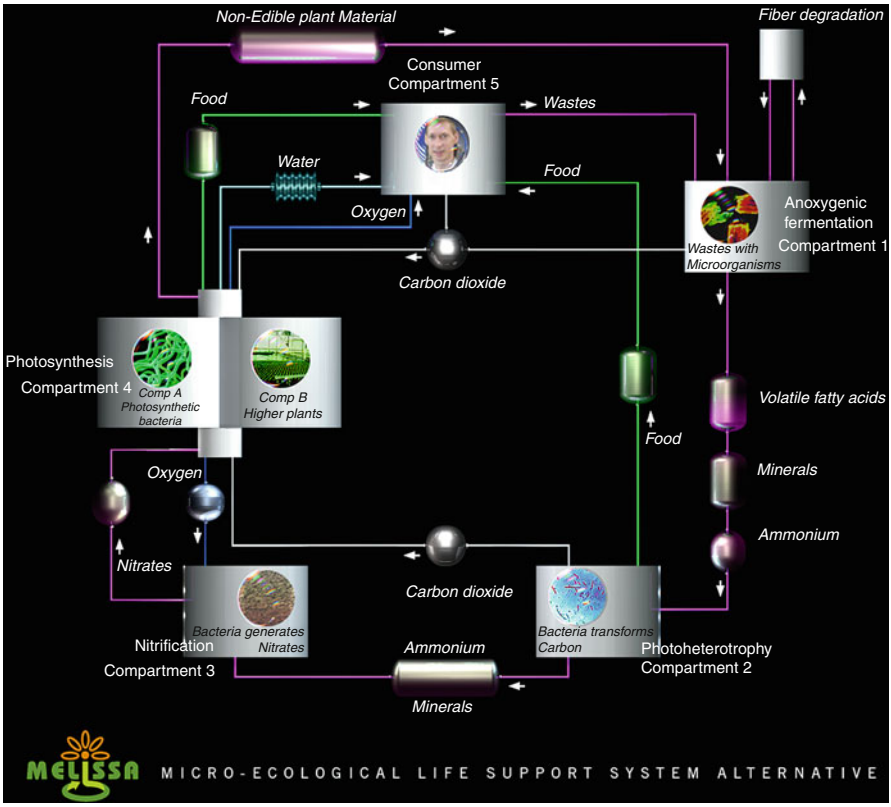

Fig. 33.3 The MELISSA advanced loop concept, showing the different compartments and their connections, forming an artificial micro-ecosystem (image ESA)

On the planetary surface, even if the soil would not contain toxic materials, the mere inhalation of dust particles, particularly in a reduced gravity environment, can pose health hazards. Free radicals, salts and oxidants can be aggressive both for human tissue and for equipment, in particular in humid conditions. Toxic materials and potential organics add to the dangers. In extremis, even unknown biohazards may be encountered such as viruses, yeasts or bacteria. New monitoring and protection techniques will have to be developed to keep such risks under control.

33.3.4 Permanent Lunar or Martian Base

Space Agencies worldwide are carrying out detailed design studies on the architecture of a permanently inhabited base on the Moon. Many important milestones are still to be reached, in particular related to radiation protection, habitat design and supply possibilities. It is certain that these will need further study, including looking into possibilities of subsurface construction and using local resources for supply (see below).

Fig. 33.4 The MELISSA pilot plant at the University Autònoma of Barcelona (image ESA)

Nevertheless, like for a human mission to Mars, the technological solutions are within reach and it is more a question of finding the proper financing that will determine the timescale. Whereas at this stage these endeavours will be predominantly government funded, there are some far away commercial perspectives on the Moon (such as 3He mining for energy production), which could lead to involvement of private parties.

For Mars, the main reason for making the trip is gathering scientific knowledge. At this moment in time it is not clear whether the scientific perspectives warrant permanent human presence. On the other hand, it should be realised that for a nominal flight to Mars, the available time for research on the surface cannot be changed at will. It is either a roughly 1 month, or almost 1.5 years, determined by the relative position of Earth and Mars allowing for a Hohmann transfer orbit. In the first case the amount of time spent on the Martian surface is less than 10% of the total mission duration, which can be considered wasteful. On the other hand, the second scenario will require something that could easily be described as a permanent base. Almost certainly the first flight to Mars will be of the first type. However, if the results from the first mission would warrant further flights to Mars, a permanent human base on Mars could become the more economical solution.

Fig. 33.5 Mars Express image, showing water ice in an impact crater on Vastitas Borealis, an area near the Martian Polar regions (image ESA)

33.3.5 Living of the Land

The greater the distance, delta-v and mission duration, the more supplies will have to be carried along. Some gains can be made by recycling technologies such as described above. However, major reductions in launch mass could be achieved if part of the consumables could be found at the place of destination. Possibilities include:

- Building material. Lunar or Martian regolith can be used, either in its pure state or after processing, as material for covering habitation modules for protection against radiation or meteorites. More advanced use could include regolith-base concrete for construction purposes. Local soil may also be used as substrate for food production (Lytvynenko et al. 2006).
- Water. The presence of water ice in Martian Polar regions has been assumed from Mars images such as provided by the ESA Mars Express mission (Fig. 33.5) and proven in 2008 by the NASA Phoenix Lander (Smith et al. 2009), whereas other underground reservoirs of (frozen) water are likely. On the Moon, it is hypothesised that water ice may be found in chasms near the Poles as well, although this is not proven.
- Oxygen. Whereas pure oxygen cannot be found on either Moon or Mars, it can be produced relatively easily from water using electrolysis. In absence of water, oxygen may also be produced from carbon dioxide, which is relatively abundant in the Martian atmosphere, or from the regolith itself. In the latter case, significant amounts of energy will be required, however (Zubrin et al. 1997). In order to reduce weight and energy requirements further (also in the sense of allowing less sturdy and hence massive constructions) it is not unlikely that early habitats will use hypobaric conditions which in itself may impact physiological adaptation and health risk (see Chap. 13).

Table 33.3 Performance characteristics of some current and future propulsion systems

Propulsion type	Specific impulse (s)	Thrust/weight
Chemical	200–400	0.1–10
Ion propulsion	1200–5000	10^{-4}–10^{-3}
Nuclear fission	500–3000	10^{-4}–10
Nuclear fusion	10^{4}–10^{5}	10^{-5}–10^{-2}
Antimatter annihilation	10^{3}–10^{6}	10^{-3}–1

- Propellant. The mass of propellant required for the return trip to Earth is quite significant. If one bases oneself on a simple hydrogen-oxygen engine, in particular, the mass of the oxygen will be important. As seen in the previous section, production of oxygen is feasible on Mars and maybe on the Moon as well. More advanced ideas exist as well, including producing methane and oxygen from Martian carbon dioxide and hydrogen.
- Metals, plastic, etc. Various studies have looked into chemical processes to produce iron, aluminium, copper and even ethylene from locally available resources. Given the presence on Mars of carbon and hydrogen, Earth-based technologies could be applicable there (Zubrin et al. 1997). On the Moon this may be more complicated, in view of the low abundance of these two elements.

The more complex the above procedures become in general, the more energy intensive they will be. Therefore, energy production, either Solar or Nuclear energy, will be an issue that needs to be resolved first. Another aspect that requires consideration is that if mission-critical consumables are supposed to be generated in this way, it is most likely wise that a mission be split in two parts, and that the human crew will only be sent on its way when positive confirmation has been obtained that the required oxygen, propellant, etc. is already available.

33.3.6 Nuclear Propulsion

To a large extent, the discussions before on the possibility to reach Mars or other destinations were based on current propulsion technologies, which are basically chemical in nature. Using different, more efficient propulsion techniques, trajectories could be chosen that are faster than the Hohmann transfer orbit, since the higher energy requirements could be met without serious mass penalties. The two key parameters to define a propulsion system are the thrust (in Newton) and the so-called specific impulse I_{sp} (in seconds), representing the achieved impulse per unit of weight of propellant. Specific impulse determines how efficient a propulsion system is (the higher I_{sp}, the less propellant is required to achieve a certain delta-v), the thrust determines how fast such delta-v will be achieved. In Table 33.3, some examples are given of I_{sp}, and thrust (or rather thrust per weight) can be achieved with current and future propulsion systems (Mallove et al. 1989; Sutton 1992).

From the table it can be seen that chemical propulsion is the most reasonable solution today, even if it is not very efficient. Ion thrusters, in which ionised heavy

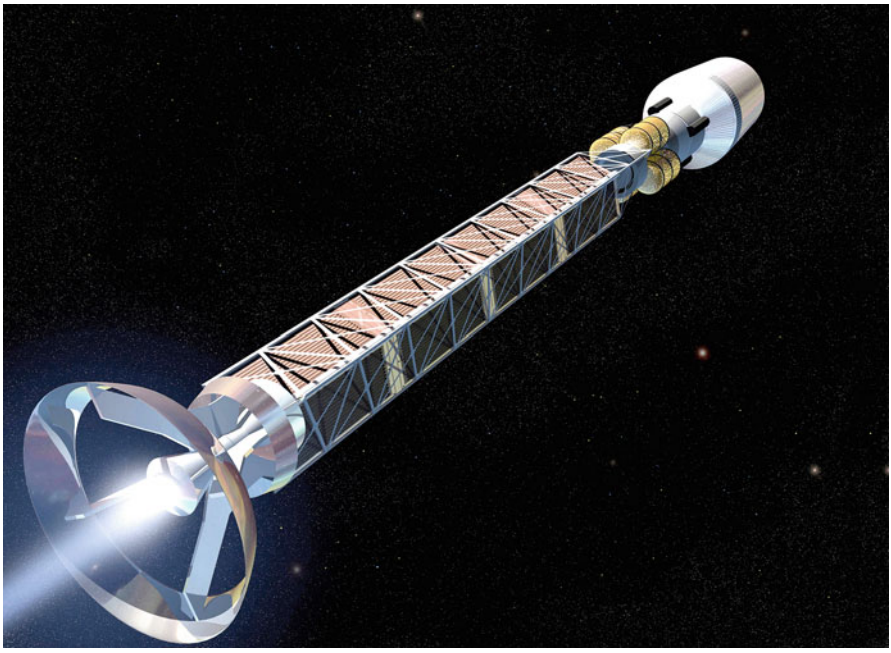

Fig. 33.6 Artist impression of a rocket using antimatter propulsion (source: Wikimedia)

gases (most often Xenon is used) are accelerated in an electric field, are much more efficient, but have very low thrust and are therefore not practical fur human spaceflight purposes. As an example, the ESA Smart-1 mission in 2004 used only ca. 60 kg of Xenon to reach the Moon from LEO, but it took 409 days to get there (Rathsman et al. 2005).

Future propulsion systems for human spaceflight will therefore be based likely on nuclear technologies. The first concrete designs for this were in the late 1940s, and assumed a series of nuclear bombs to be detonated some 50 ft behind the rocket, with the blast being caught by a pusher plate (Dyson 2002). Since then, more elegant designs have become available with less obvious drawbacks. These designs are based on nuclear fission reactors in which the energy is either used to generate power for electric propulsion or transferred to inert gas that is used as propellant (Bruno 2008). Designs on nuclear fusion are also proposed.

Even more promising, on paper at least, are engines in which anti-protons (such as are generated for example in CERN), would be stored in magnetic flasks and brought to annihilation through contact with normal matter. The energy released in such a process is enormous and can be used to generate huge exhaust velocities at the engine nozzle (Forward 1985). Designs for such engines do exist (Fig. 33.6) and prototype antiproton storage systems are in existence. Nevertheless, such systems will require amounts of anti-protons that are beyond the current capabilities, and it cannot be expected that within this century this technology will become mature.

33.3.7 Hibernation

Even when much more efficient engines would be produced routinely, the time of a space trip will remain enormous. The travel time (one way!) to the nearest neighbouring star to our Solar system, in the Alpha Centauri system at a distance of more than 4 light years, would take several tens of years in the most optimistic of all cases. If humans would ever like to become 'travellers of the universe', we will have to devise ways to extend our lifetime and overcome boredom during such trips. Many science fiction movies incorporate 'hibernation', putting the body for a long period of time at an extreme low metabolic rate, as a solution to this problem. Is this realistically conceivable? In fact, space agencies including ESA and research institutes are doing some advanced studies in this domain. Specific chemicals triggering hibernation have been identified and synthetic derivatives have been shown to be effective in non-hibernating animals (Oeltgen et al. 1988; Vecchio et al. 2003; Ayre et al. 2004). Also exposing animals to low concentrations of H_2S have been shown to lower body core temperature and metabolic rate (Blackstone et al. 2005). Clearly, further studies in this domain are mandatory before conclusive results and practical applications can be reached. This field of research is not only interesting for space exploration, but is also attracting high attention from the medical research community (Hamid et al. 2009), in view of the potential advantages for anaesthesia. It is quite possible that these terrestrial applications will drive progress in this field.

33.3.8 Terraforming

An even more drastic approach towards using the Martian environment would be the creation of a breathable atmosphere and Earth-like water-based ecosystem (McKay and Marinova 2001), in which humans could live without spacesuits or other protection, and where standard food production techniques can be employed.

A first step in this process would be raising the Martian temperature. On Mars, solid carbon dioxide is present at the poles. It can be shown that raising the temperature by only a few degrees could lead to a runaway effect, starting with the release of gaseous carbon dioxide which in turn, through the greenhouse effect, will lead to higher temperatures and release of even more carbon dioxide. In this way, the surface temperature on Mars could approach the melting temperature of water. There would be several ways to achieve the initial raise of temperature required for this process. Under more or less serious consideration are the use of large Solar mirrors, redirecting meteors towards Mars and using the energy released on their impact, and adding strong greenhouse gases like CFC's to the Martian atmosphere. Needless to say all of these solutions require massive technological developments and will not be available in this century.

A next step in terraforming Mars would be the activation of an artificial ecosystem. Suitable bacteria, most likely selected extremophiles, could initiate this, followed by plant life once sufficient water and organics will be available. This will then raise the oxygen concentration in the atmosphere, further increase of the temperature and

finally the creation of a more or less Earth-like environment. It is estimated that the total process will require hundreds to thousands years as a minimum.

Of course, such a drastic development is at odds with current ethical values and principles of planetary protection, which say that mankind should leave other planetary systems pristine and free from Earth contamination.

33.4 Summary

The purpose of this chapter has not only been to list the challenges and caveats but to demonstrate also that human space travel beyond Low Earth Orbit seems possible. For the more immediate destinations, Moon and Mars, the scientific and technological knowledge of today is very likely sufficient to start planning such a mission. Obviously, further research and understanding is needed on how in the most complex biological system man will cope with space environment for very extended periods and how he can – also supported by countermeasures – overcome the suffering from allostatic (over-)load. Much research, testing and technological progress will be required to make this practically (and financially) realisable.

At the same time it is interesting to see that current research points out ways to expand human presence to further distances and/or permanent settlements. Although some of these ideas sound still very fantastic, creative minds are obviously busy to push the boundaries. History has demonstrated that humans are willing to take large risks to explore new horizons. It seems therefore inevitable that a new Columbus (or, more likely, a huge team of them) will emerge and take off for this new voyage. The question is hardly 'how,' but 'when.'

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