Human Research Program
Human Health Countermeasures Element

Evidence Book

Risk of Crew Adverse Health Event Due to Altered Immune Response

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I. PRD Risk Title: Risk of Crew Adverse Health Event Due to Altered Immune Response

Description: Human immune function: Human immune function is altered in- and postflight, but it is unclear if this change leads to an increased susceptibility to disease. Reactivation of latent viruses has been documented in crewmembers, although this reactivation has not been directly correlated with the immune changes or with observed disease. Further research may elucidate whether microgravity exposure impairs the immune system, and whether this change represents a health risk to crews.

II. Executive Summary

There is a large body of evidence associated with immune dysregulation and spaceflight; however, the current evidence base is insufficient to determine clinical risk related to immune dysregulation during exploration space missions. Evidence is lacking primarily because precious little of the current information is derived from in-flight studies with human subjects. The in-flight studies that have been performed have used extremely small numbers of subjects or been limited to short-duration flight. There is more data derived from postflight testing of crew members, but these findings do not necessarily reflect the in-flight condition. Rather, landing-day observations may be skewed by the effects of re-entry and readaptation to gravity following deconditioning. Ground-analog testing of humans, while extremely useful for some applications (assay development, countermeasures validation, etc.), can never be said with certainty to mirror physiological changes during spaceflight. Each analog may simulate some aspect of flight, but no analog can cumulatively replicate all the aspects of flight.

In-flight testing of humans has revealed that latent herpes viruses reactivate to a high level during short-duration spaceflight, but it is currently unknown whether this phenomenon would persist or intensify during extended duration flight, or eventually resolve itself. During long duration flight, cell-mediated immunity has been demonstrated to be reduced in some subjects, and there may be a relationship between the observed in-flight immune changes and reactivation of latent viruses. Postflight human testing has revealed severely depressed T cell function following 6 months of flight, but unaltered function following short-duration flight. Altered cytokine production patterns and potentially a shift to the Th2 pattern have been observed following spaceflight. Natural killer (NK) cell, monocyte, and neutrophil function have all been found to be reduced following spaceflight. Stress hormone levels have been found to be elevated following flight, heavily dependent on mission duration. Various animal studies have demonstrated similar findings either during or after flight.

Human ground analog data have varied widely depending on the analog selected. To date, the 6-month Antarctic winter over appears to be the best terrestrial analog for long-duration spaceflight relevant to immunity. Reactivation of latent herpes viruses, immune dysregulation, and physiological stress that appear similar to spaceflight-associated immune dysregulation have all been observed in the Antarctic analog. Shorter-duration analogs such as the undersea NEEMO missions (off Key Largo, 2 weeks duration) and Arctic expeditions on Devon Island (Haughton-Mars Project, ~1.5 month's duration) also appear to be promising analogs for short and intermediate duration spaceflight respectively. Recent pilot studies have found immune dysregulation similar to that associated with spaceflight during both of these analogs, and both

are far easier to use, from a logistical perspective, than Antarctic winter-over. Bed rest has been reported by some investigators to induce immune changes, but the contrary has been reported by other investigators. While prolonged bed rest is an excellent analog for bone loss and muscle deterioration from lack of use, it does not simulate the primary suspected causes of spaceflight-associated immune dysregulation (including physiological stress, disrupted circadian rhythms, and microgravity).

Terrestrial clinical research has repeatedly demonstrated that altered immunity is associated with adverse conditions, and that immune "balance" is essential for good health. For example, depressed immunity may lead to increased incidence of infections, but elevated immunity may lead to allergies or autoimmunity. More specific alterations in Th1/Th2 cytokine balance may be associated with many conditions, including rheumatoid arthritis, multiple sclerosis, asthma, lupus, and allergies. If immune dysregulation were found to persist in the deep space environment, clinical risks could include hypersensitivities, allergies, autoimmunity, increased infection rates and even malignancies associated with impaired tumor surveillance. Rather than develop gradually, the risks related to altered immunity have the potential to suddenly impact a mission, likely in a manner very difficult to treat remotely. The reactivation of latent viruses, while not typically a clinical concern terrestrially, could pose a health risk if persisted for the duration of an exploration class mission.

Given the widely disparate nature of the immune evidence and the huge knowledge gap related to in-flight testing of human subjects, it cannot be determined at this time if significant immune dysregulation persists or resolves during extended duration spaceflight. This argues strongly for a comprehensive in-flight assessment of immunity, viral reactivation and physiological stress. It is intended that the new multi-laboratory *Integrated Immune (SMO-015, SDBI-1900)* flight study will provide sufficient in-flight long-duration data during the International Space Station (ISS) utilization phase to allow a determination of clinical risk (if any) related to immunity for exploration class missions. Other new studies, such as the European Space Agency's *Immuno* experiment (ISS/Soyuz), will also add to the in-flight knowledge base.

III. Introduction

The assessment of immunity is a fast-developing, ever-changing area of science, made difficult by the complexity of the immune system. A number of distinct subpopulations of leukocytes (white blood cells) populate the blood, lymph nodes, and gut, and generally traffic around the entire body. The specific functions of these cell populations can vary widely, and in some cases they are counter regulatory. In addition, the emerging science of neuroimmunology illustrates the complexity with which the immune system interacts with other physiological systems via a network of communication involving hormones, cytokines, and cells. That altered immunity is directly related to the presence of disease is well-accepted and may be clinically related to increased incidence of infection and increased risk of tumor formation. For this report, immune dysregulation is defined as a deviation from "normal," or from pre-flight baseline values. Dysregulation detected by individual immune assays may be either hyper-activity or hypo-activity. Clinically, hyper-immunity is associated with allergies and various auto-immune diseases, whereas hypo-immunity can be associated with increased incidence of infection and possibly tumor formation. In addition, the balance and bias of the immune system *within itself* (Th1/Th2) is correlated with risk and incidence of specific diseases.

To advance the study of immunity, a large number of assays have been developed. In the hospital laboratory, determination of the number of T cells positive for the surface protein CD4 (CD4+) and the titer of antibodies to viruses are well-established tests utilized in clinical practice. However, there are dozens of other direct measurements of specific immune parameters available for clinical research. Examples of immune assays are measurement of the level of immune cell subpopulations in the blood (phenotyping), isolation and stimulation of cultured immune cells followed by various functional assessments, determination of factors such as mRNA gene expression, secretion of cytokines, and expression of activation markers.

Published data strongly suggest that immune dysregulation is associated with spaceflight, regardless of duration, and several excellent reviews have been published recently regarding this subject (Borchers 2002, Sonnenfeld 2002). The specific single cause of immune dysregulation during flight is unknown, but likely associated with one or more of the following items: physiological stress, disrupted circadian rhythms, microgravity, isolation, altered environment, altered nutrition, and radiation. While the postflight status of the human immune system has been well characterized, the status of the immune system during flight (and particularly during longer duration flight) is incomplete. A general overview of spaceflight-associated immune dysregulation (potential causes, summary of observations to date, and potential clinical risk) is presented in figure 13-1. A current goal of NASA and the space life science community is to determine the specific clinical risks associated with all flight effects on human physiology, so that countermeasures may be developed prior to the initiation of exploration-class space missions. This need has been heightened by the impending NASA lunar/Mars program, which will be initiated within the next decade.

The published data regarding spaceflight and immunity may fall into numerous categories: during flight, after flight, ground analog, human, animal, and so on. For astronaut crewmembers, data are typically captured as follows: before flight to establish baseline values, during flight (when possible), and on landing day (R+0) to establish spaceflight associated changes, and after flight to monitor return to baseline. Due to the limits of practicality, it is impossible to review ALL the evidence. For this report, numerous representative articles have been summarized by category in Appendix 1. These articles reflect the primary scientific findings for the discipline. The narrative text of this report represents a summation of the data and likely clinical significance.

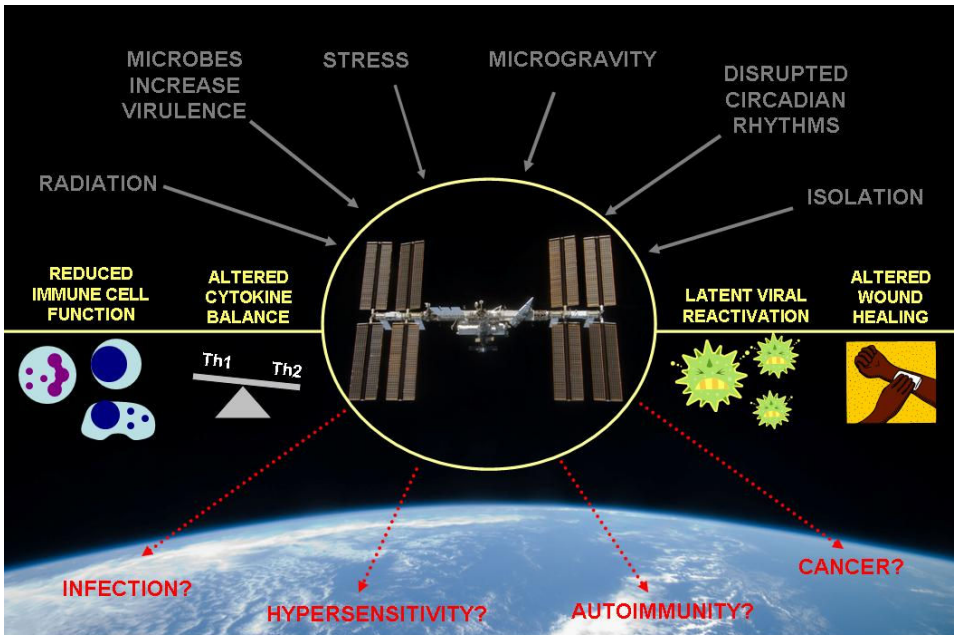


Figure 13-1. Overview of spaceflight-associated immune dysregulation.

IV. Evidence

A detailed presentation of representative published articles regarding various aspects of space-flight immunity is presented in Appendix 1. The narrative description of evidence represents a non-comprehensive summary of the published evidence.

A. Spaceflight Evidence

1. In-flight Human Data

Due to the complexities associated with in-flight experiments, there have been comparatively few *in-flight* studies of human immune function. Those that have been performed have weakened by having a small number of subjects (n). Reactivation of latent herpes viruses has been observed repeatedly during short duration flights (Mehta/Pierson 2000, Payne 1999, Pierson 2005, Stowe 2001, Mehta 2004), and depressed cell mediated immunity (CMI), determined in vivo using the CMI skin test, has been observed during long duration flight (Cogoli 1993, Gmunder 1994).

Recently, the in-flight reactivation of latent herpes virus during a short-duration Soyuz taxi flight to the ISS was assessed (Mehta 2007). Three crewmembers who participated in a recent 14-day mission to the ISS were monitored for the reactivation of varicella-zoster virus (VZV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV) using blood, urine, and saliva assays. During flight, only saliva was assayed; urine and blood were tested before and after flight. The data demonstrated EBV reactivation before, during and following flight. CMV was reactivated before and after flight, and VZV was reactivated during and after flight (Figure 13-2). No increases in titer of antibodies to these viruses were found, suggesting that an immune response may not be necessary for reactivation to occur. These data support the Shuttle findings, in that similar changes were found following short duration flight on different vehicles. No in-flight data exists regarding latent viral reactivation during long-duration flight.

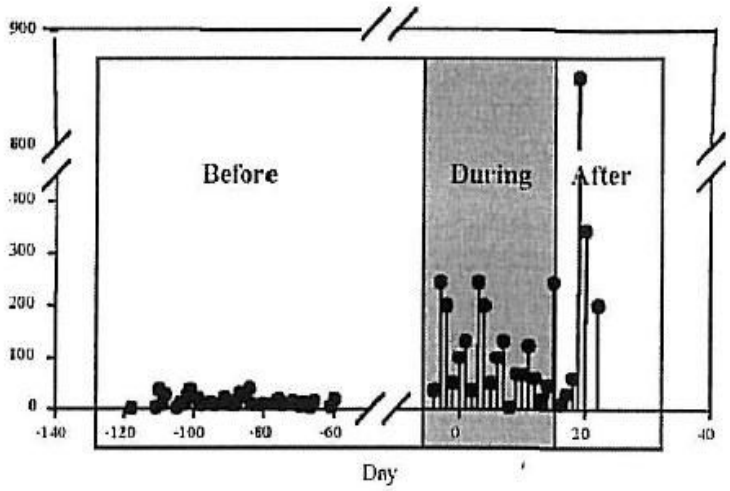


Figure 13-2. VZV DNA shedding in three crewmembers before, during and after a Soyuz taxi mission to the ISS.

Unfortunately, there has not yet been an integrated in-flight study assessing the various aspects of human immunity during long-duration space missions. The current European Space Agency (ESA) in-flight "*Immuno*" study (long duration, ISS/Soyuz, n=6) and the NASA in-flight "*Integrated Immune*" study (long duration, ISS/Shuttle, n=17) will address this in-flight knowledge gap and allow a determination of clinical risk (if any) associated with immunity during prolonged exploration class flight.

2. Unpublished In-flight Incidence Rates

In addition to the experimental evidence, with regard to in-flight testing of human subjects there is also substantial anecdotal data regarding immunity during flight. This is almost entirely clinical and consists of crew reports regarding adverse medical events that occurred during various space missions. Such data are potentially very useful, if they reflect consistent flight-related alterations in disease progression or wound healing. However, in accordance with Health Insurance Portability & Accountability Act of 1996 regulations, such clinical data are generally restricted and may be available for science purposes only in an un-attributable fashion. Recently, Dr. Kathy Johnson-Throop of the JSC Life Sciences Data Archive surveyed the Shuttle clinical data archive for in-flight incidence rates of infectious disease. The number of events that occurred in these very healthy, screened and essentially isolated individuals was remarkable, especially in consideration of the pre-flight quarantine of all Shuttle crewmembers (Table 13-1).

Table 13-1. Shuttle incidence of in-flight infectious disease* (STS-1 through STS-108).

Number	Infectious Disease
8	Fever, chills
5	Fungal infection
3	Flu-like syndrome
4	Urinary tract infections
3	Aphthous stomatitis
2	Viral gastrointestinal disease
2	Subcutaneous skin infection
2	Other viral disease
29	**Total incidents in 106 Shuttle flights (approximately 742 flown crewmembers)**

*Based on postflight medical debriefs [Longitudinal Study of Astronaut Health] – Dr. Kathy Johnson-Throop

3. Postflight Human Data

Given the relative ease of performing postflight assessments of human immunity, a significant number of studies have been performed. Advantages of postflight evaluations include lower cost, readily available access to human participants, and minimal technical barriers. The primary disadvantage of a postflight assessment is the potential that data do not reflect the in-flight condition and are skewed by the confounding physiological stress of re-entry and re-adaptation to unit gravity; however, as an important starting point, much has been learned about crewmember immune status immediately following both short- and long-duration spaceflights.

Specific immune system alterations that have been observed when testing was done immediately after spaceflight include dysregulation of the following: cytokine production patterns (Chapes 1994, Crucian 2000, Gould 1985, Gould 1987, Konstantinova 1995, Miller 1995, Sonnenfeld 1994, 1996, 1988, 1993); NK cell function (Buravkova 2004, Konstantinova 1995, Meshkov 1995); leukocyte distribution (Crucian 2000, Stowe 1999); monocyte function (Kaur 2005, Maine 1991); granulocyte function (Kaur 2004, Stowe 1999); T cell intracellular signaling (Cogoli 1997, 1993; Pippia 1996, Schwarzenberg 1999); neuroendocrine responses (Stowe 2003); and leukocyte proliferation following activation (Grove 1995, Nash 1992).

One may briefly summarize these observations as follows: there appears to be a generalized multi-faceted immuno-suppression that is detectable postflight. Production of various cytokines (intracellular, secreted, or otherwise) is reduced, and the functional capabilities of the various immunocytes (granulocytes, monocytes, NK cells, T cells) are reduced. Although some of these findings are almost certainly related to landing and readaptation, more recent findings indicate at least some postflight observations may reflect in-flight immune alterations. Specifically, when expression of EBV early, intermediate, and late genes was measured in infected peripheral B cells, expression of EBV late genes was elevated in samples collected immediately postflight (see unpublished postflight data: EBV gene expression).

Although latent herpesvirus reactivation has been found to occur to high levels during short duration space flight, some had speculated that the salivary measurements of viral DNA might not reflect infectious viral particles. To investigate this further, following a recent Space Shuttle mission, cultures were performed to determine if infectious virus was being shed. In 2 of 3 astronauts who participated in the study, following landing live VZV was found to be present in the saliva samples (Cohrs 2008).

Recently, a comprehensive post-flight immune assessment was performed on 17 short-duration Space Shuttle crewmembers and 8 long-duration International Space Station (ISS) crewmembers (Crucian 2008). The testing consisted of a comprehensive peripheral leukocyte subset analysis, determination of early T cell functional capabilities, and intracellular/secreted cytokine profiles. For Shuttle crewmembers, the distribution of the peripheral leukocyte subsets was found to be altered post-flight. Early T cell function following culture stimulation was actually elevated post-flight; however, the percentage of T cell subsets capable of being stimulated to produce IL-2 and IFNg was decreased. The ratio of secreted IFNg:IL-10 following T cell stimulation declined after landing, indicating a Th2 shift. For the ISS crewmembers, some alterations in peripheral leukocyte distribution were also detected after landing. In contrast to Shuttle crewmembers, the ISS crewmembers demonstrated a statistically significant reduction in early T cell activation potential immediately post-flight (figure 13-3). The percentage of T cells capable of producing IL-2 was reduced, but IFNg percentages were unchanged. A reduction in the secreted IFNg:IL-10 ratio (Th2 shift) was also observed post-flight in the ISS crewmembers.

These data indicate that consistent peripheral phenotype changes and altered cytokine production profiles occur following spaceflight of both short and long duration; however, functional immune dysregulation may vary related to mission duration. A more pronounced T cell dysfunction was observed in the ISS crewmembers, indicating that prolonged mission duration and deconditioning may affect immunity. The elevations in function in the Shuttle crewmembers were attributed to the acute stress of landing, in much less deconditioned subjects. Finally, the authors conclude that a Th2 cytokine shift appears to be associated with spaceflight. This potentially indicates specific classes of clinical risk (autoimmunity, hypersensitivities), should such shifts persist for exploration-class space missions. These data were derived from the *Epstein Barr* flight study (DSO-500, E129).

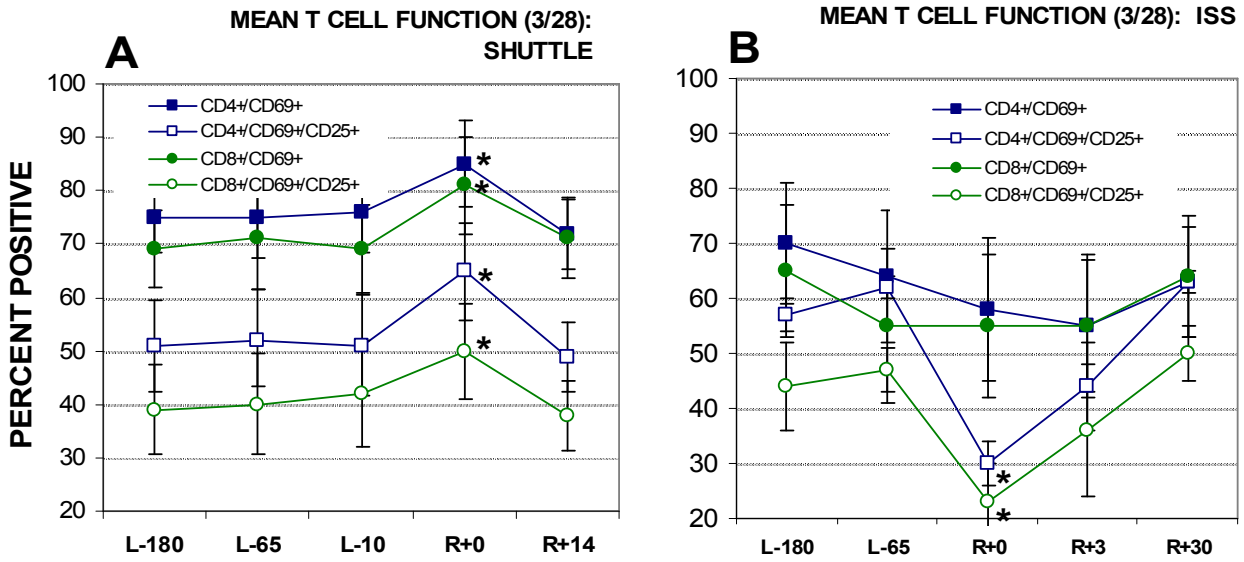


Figure 13-3. T cell function was elevated immediately post-flight for short-duration Shuttle crewmembers (A), but was significantly reduced post-flight for long-duration ISS crewmembers (B). Data were derived from culturing whole blood with antibodies to stimulate T cell activation for 24 hours, then assessing T cell activation marker expression (CD69 or CD25). Number of subjects to date: shuttle = 9, ISS = 6.

4. Unpublished Postflight Data: EBV Immunity

EBV-specific T cell immunity, responsible for controlling viral reactivation, is altered following spaceflight. Representative crewmember data are presented in Figure 13-4. Levels of EBV-specific T cells (measured by the MHC tetramer method) rise following flight, likely indicating an attempt to control latent virus reactivation. However, EBV- specific T cell function (IFNg cytokine expression following EBV peptide stimulation in culture) was dramatically reduced following spaceflight. These data (derived from the *Epstein Barr* flight study [DSO-500, E129]) indicated that the immune defect that allows viral reactivation to occur is altered T cell function, not a loss of specific T cells responsible for control of virus. Prolonged similarly reduced function of the entire T cell population could result in immunosuppression and disease susceptibility.

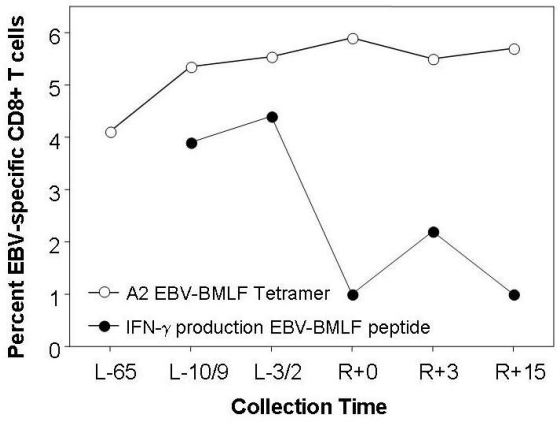


Figure 13-4. Representative data from a single short-duration crewmember demonstrating an increase in levels of virus-specific T cells (open circles, tetramer method) simultaneous with a reduction in their functional capabilities (dark circles, IFNg production after stimulation with viral peptide).

5. Unpublished Postflight Data: EBV Gene Expression

The level of EBV mRNA gene expression in infected peripheral B cells was found to be altered following spaceflight compared to both the pre-flight baseline and normal healthy controls. Several EBV genes were measured by quantitative polymerase chain reaction (PCR); they were subdivided into genes expressed during EBV latency, genes expressed intermediate-early or early (IE/E), and genes expressed late in the active replicative phase of infection and reactivation. For this assay, actin mRNA was measured as a positive control and the EBV EBER gene was measured as a control for the presence of infected B cells. Transcripts of the EBER gene are expressed to varying degrees in all infected B cells, regardless of the viral latency state.

EBV gene expression in peripheral blood from healthy adults is highly restricted. Five of 24 samples were positive for latent gene expression, while only 3 of 24 samples were positive for IE/E expression; none of the 24 control subjects were positive for any of the late replication EBV genes. For Shuttle crewmembers, 5 of 12 samples showed evidence of latent gene expression, while nine of 12 samples had IE/E gene expression. Notably, some of the samples were positive for multiple viral gene transcripts. None of the pre- or postflight samples were positive for late gene transcripts.

For ISS crewmembers, nine of 12 samples were positive for latent gene transcripts, while 11 of 12 were positive for IE/E gene transcripts. In addition, a much higher percentage of samples were positive for multiple viral gene transcripts. Importantly, for the first time, evidence of late (i.e., replicate) gene transcription was found, postflight only: four of the six landing day samples were positive for one or more of the late genes. None of the samples from healthy controls or the Shuttle postflight samples demonstrated any late EBV gene expression. Note that for the astronaut crewmembers, all subjects demonstrated EBER expression; thus the actin control is not shown. These data (derived from the "*Epstein Barr* flight study [DSO-500, E129]) suggest that reactivation of latent EBV is to some degree observed at the L-10 postflight timepoint. However, it is known that for some stress measurements, L-10 may be too close to launch to serve as a fair baseline as crewmember stress is present by this point in the countdown. Also, the data show that EBV reactivation is affected by mission duration. This may be due to poorer cellular immune control over viral replication during prolonged space missions. Alternatively, spaceflight may have a more direct effect on viral reactivation and replication. Persistent reactivation of latent herpes viruses during long duration space missions, in conjunction with dysregulated immunity, high energy radiation, and other factors, may represent

a significant clinical health risk to crewmembers participating in exploration-class deep space missions. (Publication anticipated 2009)

6. In-flight Animal Data

Unfortunately, the majority of spaceflight immunity studies with animal subjects have involved postflight testing of animals flown in space. Those studies are listed under "postflight animal data." Some animal studies have assessed immunity during spaceflight; these primarily involved dissection of animals during flight and return of samples to earth. On the Shuttle SLS-2 mission, rats were flown and samples returned for analysis. Spleen T cell and NK cell function were reduced during and after flight; however, marrow NK cells appeared to be unaltered. Altered cytokine production patterns were also reported (Lesnyak 1996).

7. Postflight Animal Data

A number of animal studies during spaceflight have provided biosamples available for postflight testing. In particular, immune splenocytes and thymocytes have been available from rats and mice flown in space on several SLS Shuttle missions. On several flights of Russian COSMOS satellites, live rats were flown and recovered. Several variables in these studies cannot be controlled, such as mission duration, differing launch vehicles, and different animal species. These additional variables make comparison of data from different studies difficult; however, actual flight data remains extremely valuable, regardless of vehicle.

The postflight animal data include observations of altered leukocyte distribution (Sonnenfeld 1990, 1992) and altered cytokine production (Gould 1987, Grove 1995, Miller 1995, Sonnenfeld 1996). One study indicated that postflight mitogenic and proliferative responses of lymph node lymphocytes, as well as IL-2 production, were unaltered in space-flown rats (Nash 1992). In general, the animal data are similar to the postflight human data: immune dysregulation is observed post spaceflight. Changes include dysregulated cell function (proliferation, cytokine production, and other functions). Interestingly, Nash et al. suggested that microgravity has a tissue-specific effect on lymphocyte function, a finding that is impossible to evaluate in human subjects and highlights the greater utility of animal studies for in-flight immune investigations.

Recently, mice flown on the STS-118 Space Shuttle mission were available for immunological studies. For this study, spleens and thymuses from flown mice were evaluated in comparison to similarly held ground controls. Samples were collected 3-6 hours following Shuttle landing. In general, the observations were similar to those obtained from human subjects. Alterations in the distribution of the lymphocyte subsets, a reduction in blastogenesis following mitogenic stimulation, and shifted cytokine profiles. Specifically, IL-2 production was decreased, whereas IL10 and IFNg were increased. In addition, alterations in the expression of 30 cancer-related genes was reported (Gridley 2009). During the same mission, innate immune function was investigated, by determining responses to LPS stimulation. Secretion of IL-6 and IL-10, but not that of TNFa was increased in the flown mice as compared to ground control mice (Baqai 2009). Also, the genes responsible for scavenging ROS were upregulated.

8. In-flight Cell Culture Data

Several studies involving in-flight culture of immune cells have been performed. These data should be interpreted with caution, as it is unknown if in-flight culture observations accurately reflect altered (or unaltered) in vivo human immune function. Specific findings include: unaltered NK function (Buravkova 2004), altered cytokine production profiles (Chapes 1994), and various observations of altered progression of cellular activation following mitogenic stimulation (Hughes-Fulford 2001 and 2003, Cogoli 1993 and 1997, Hashemi 1999).

B. Ground-based Evidence

1. Ground-analog Human Data

To evaluate the effects of mission-associated factors on human physiology, ground-based "spaceflight analogs" may be used (Schmitt 1993). A variety of analogs are available, each unique and exerting some influence on human physiology that is similar to one or more aspects of spaceflight. Examples of the most well-known human ground-based spaceflight analogs are presented in Table 13-2.

Table 13-2. Human analogs for spaceflight.

ANALOG	SPACEFLIGHT RELEVANCE
Extended head-down bed rest	Fluid shifts, bone demineralization, muscle loss
Closed-chamber confinement	Psychological and isolation issues
Haughton-Mars Project (Arctic)	Isolation, extreme environment, circadian rhythms
NEEMO (undersea station)	Isolation, real mission activities, risk, EVA, extreme environment, circadian rhythms
Antarctic winter-over	Isolation, confinement, extreme environment, circadian rhythms, physiological stress, long duration

In addition, analogs may be augmented. For example, a recent study at Brooks Air Force Base by Dr. Raymond Stowe added high-G human centrifugation prior to and after a 16-day prolonged head-down tilt bed rest, to simulate launch and landing and thus better replicate the physiological aspects of a shuttle mission (Stowe 2008). Another recent study by Dr. Bill Paloski added a daily 1-hour 2.5xG human centrifugation to a 16-day bed rest study, to evaluate artificial gravity as a possible countermeasure. For ground-based studies, it is very important to choose the analog that is most appropriate for the physiological system of interest. For example, bed rest may be an excellent analog for muscle loss, whereas NEEMO or Antarctic winter over would not, since the prime causal factor (microgravity) is not replicated.

Since analog use is comparatively cost-effective, the immunology discipline at NASA is pursuing validation of an appropriate ground-based spaceflight analog for spaceflight-associated immune dysregulation. This pursuit is based on suggestions made at the 2006 Immunology Program Review and on consensus direction statements provided during the 2007 NASA Human

Research Program Workshop. Validation of a ground-based analog would be extremely useful for basic science as well as countermeasures validation, in the event that spaceflight-associated immune dysregulation is found to persist during long-duration flight and countermeasures development is warranted. An excellent ground-based flight analog for immune studies would simulate mission-associated stress, isolation, and disrupted circadian rhythms. An overview of immune data collected during various analog studies follows.

Antarctic Winter-over Analog. During Antarctic winter-over (AWO), subjects experience prolonged isolation in a harsh extreme environment, and several comprehensive immune studies have been conducted during these expeditions. It is likely that AWO represents the closest analog to long-duration or exploration class spaceflight available on Earth. This is because the mission length, extreme environment, extreme isolation, mission associated activities, disrupted circadian rhythms, and other factors are similar to those of long-duration spaceflight. During AWO, participants are completely isolated, as no aircraft are capable of reaching the various Antarctic outposts during this time. Immune studies performed during winter-over missions have shown decreased cell mediated immune responses (Muller 1995a, 1995b, Mehta/Lugg 2000), reduced T cell function (Tingate 1997), altered cytokine production profiles (Shearer 2002, Tingate 1997) and latent herpesvirus reactivation (Mehta/Lugg 2000, Tingate 1997). A study of antibody production following immunization during AWO found no mid-mission alterations (Shearer/Reuben 2001), potentially indicating that humoral immunity is unchanged in the presence of altered cellular immunity. These data support the Antarctic analog as the most successful to date in simulating long duration spaceflight-associated immune dysregulation. The only serious limiting factor regarding physiology studies during AWO, is the logistical access during a mission. Ironically, this is directly related to the very factors that make AWO such a good analog, thus truly making it "flight-like." Studies that require simple collection and freezing of samples (blood, saliva) are obviously very compatible with mid-mission AWO studies. However, as recent data have indicated, it is immune function that appears to be compromised following (and during?) spaceflight. Assessments of immune function typically require live cell culture and more immediate processing and analysis of samples. This difficulty was addressed during preparation for the Haughton-Mars 2001 immune pilot study (Crucian 2007).

Haughton-Mars Project Analog (Canadian Arctic). Another potentially useful analog for spaceflight associated immune dysregulation is the NASA Haughton-Mars Project (HMP). The HMP is an international field research project centered on the scientific study of the Haughton meteorite impact structure and surrounding terrain on Devon Island, Nunavut Territory, Canadian High Arctic. It is viewed as an analog for planetary exploration, in particular for exploration of the Moon and Mars. It is particularly well suited for exploration-related human physiology studies because field personnel are subject to actual *and* relevant environmental stressors, although they are clearly not as extreme as those encountered in space. In addition, personnel are engaged in field exploration tasks that in many cases are direct analogs of those anticipated for the Moon and Mars. The following factors encountered by HMP field participants are particularly relevant to spaceflight or planetary exploration:

- Long travel to and from Devon Island (several days of travel followed by weeks of stay)
- Relatively harsh polar desert environment

- Disrupted circadian rhythms (24 hours of daylight during the summer field season)
- Relative isolation from the outside world (with limited exception)
- Limited local infrastructure (HMP Research Station is analogous to early lunar or Martian outpost)
- Activities relevant to those that crewmembers on lunar and Mars missions would be expected to perform, including exploration, field work, EVA
- Reliance on remote telemedicine and communications equipment.

These factors and mission duration make the HMP potentially a good analog for spaceflight-associated immune dysfunction studies. The 30- to 45-day mission duration seems to make HMP a potentially useful analog for ISS Flight Engineer-2 subjects, who rotate on successive Shuttle flights and have mission durations longer than those of Shuttle-only crewmembers but shorter than those of ISS-Soyuz (6-month) crewmembers. In 2002 a NASA pilot study was performed with the following objectives: (1) develop and field-evaluate methods for processing biological samples to support immune function testing in remote locations, and (2) use the protocol to assess mission-associated immune changes during an HMP mission. The data demonstrated that in the HMP participants, changes in immune function and physiological stress occurred that were in some ways similar to those observed in astronauts following spaceflight (Crucian 2007). Specifically, phenotypic alterations, reductions in intracellular cytokine levels, humoral data that suggested EBV reactivation, and altered stress hormone levels were all observed during this intermediate length HMP mission.

NEEMO Undersea Analog. A third likely relevant analog for immune changes observed during short-duration spaceflight is the NASA Extreme Environment Mission Operations (NEEMO) project. The NEEMO project was developed by NASA to use extended undersea missions based in Aquarius (the world's only permanent undersea station) as a high-fidelity ground-based spaceflight analog. Aquarius was constructed and is operated by a partnership of the National Oceanic and Atmospheric Administration, the University of North Carolina at Wilmington, and the National Undersea Research Center, and is utilized routinely for undersea oceanic research. It is located 7 miles offshore of Key Largo, Florida at a depth of approximately 65 feet. During research missions, which typically last 7-14 days, crewmembers ("aquanauts") use saturation diving. In this dive protocol, easy return to the surface is not possible and nominal resurfacing requires approximately 18 hours of decompression. NEEMO missions are timelined and executed in such a way that the spaceflight analog conditions are the best possible:

- Confinement to the station lasts the duration of the mission.
- EVA activities are performed, linked to Mission Control Center in Houston for support.
- A variety of Shuttle and ISS experiments are performed.
- Telemedicine is used to communicate with NASA flight surgeons.
- For high fidelity, only Shuttle or ISS food may be consumed (NEEMO-5).

It is important to note that although the NEEMO missions simulate high-fidelity actual spaceflight conditions, they are actual missions in their own right with real health risks and are not necessarily only simulations. Given the short mission duration and high-fidelity similarity to

a Shuttle mission, NEEMO may represent a useful analog for the spaceflight-associated immune dysregulation that has been observed during short duration spaceflight. Also, NEEMO is extremely easy to use logistically, making it an attractive test bed for hardware and initial countermeasures development. Pilot data generated during the NEEMO 4, 5, 12, and 13 missions have indicated that immune dysregulation and viral reactivation similar to those observed during or following spaceflight occur during NEEMO missions.

To investigate if NEEMO induces in-flight immune alterations similar to those observed during Shuttle missions, and to evaluate assays developed for SMO-015, a pilot study was performed on NEEMO 12 and 13, during 2007. Assays were performed that assessed immune status, physiological stress, and latent viral reactivation. Blood and saliva samples were collected at pre-, mid-, and post-mission timepoints. The data revealed minimal changes in peripheral leukocyte subsets, as would be expected from healthy subjects in an adverse environment in the absence of actual illness. Also revealed, however, were dramatic alterations in T cell function. Intracellular cytokine profiles within T cell subsets were altered, and generalized T cell function was diminished during the missions, in a fashion similar to that observed postflight in ISS crewmembers (Figure 13-5). Serological evidence of EBV reactivation was observed in 50% of the subjects. As evidence of latent VZV reactivation, salivary VZV DNA was detected in 2 of the 4 NEEMO-12 subjects. Plasma cortisol was elevated in some of the NEEMO subjects, and salivary concentrations of cortisol were greater during the mission than before or after it. Taken together, the pilot study data seem to validate the NEEMO analog as being appropriate to induce some of the aspects of spaceflight-associated immune dysregulation that are observed during short duration Shuttle flights. In addition, the ease of utility and high-fidelity of the analog make it attractive for rapid investigations. However, to investigate immune dysregulation associated with prolonged missions (a key element to determining clinical risk for exploration class missions), another analog would be required.

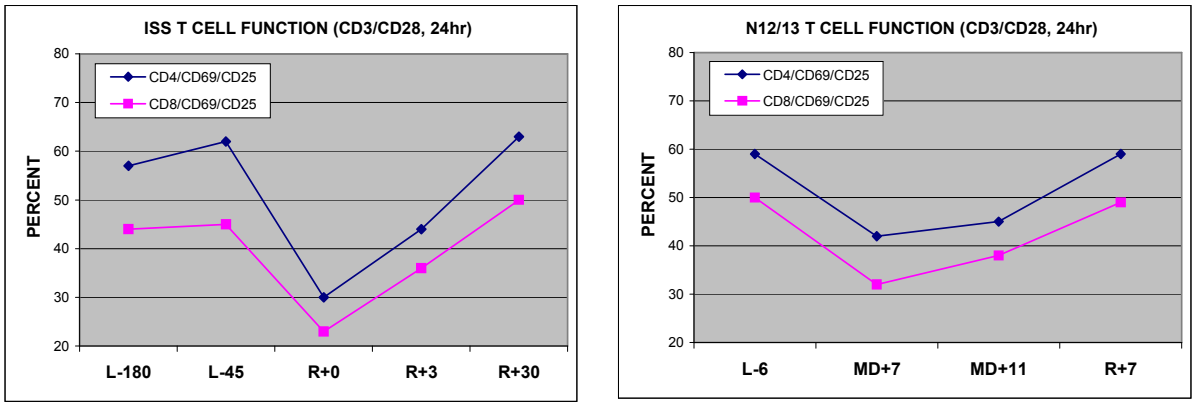


Figure 13-5. T cell function (CD69/25 expression following CD3/CD28 triggering during whole blood culture) is reduced in crewmembers following a 6-month flight (panel on left) and during NEEMO missions.

Head Down Tilt Extended Bed Rest Analog. The use of long duration head-down tilt bed rest has also been investigated to determine if this analog is appropriate for spaceflight associated immune dysregulation. The most obvious relevance for bed rest is for studying muscle loss and bone demineralization, but some investigators believe that the fluid shifts replicated during bed

rest may be relevant to in vivo immune alterations. Pieces of the evidence conflict regarding validation of the bed rest analog as a replicate for spaceflight-associated immune dysregulation. Schmitt, Uchakin and others have published data indicating that some immune changes, including decreases in T cell activation potential and altered cytokine patterns, are associated with this analog (Schmitt 1996, Uchakin 2007). However, these data were induced by exogenous delivery of stress hormones to the participants. Data generated during a general immune assessment as part of the recent NASA Flight Analogs Project bed rest campaigns did not show altered leukocyte distribution, altered cytokine production patterns, reduced T cell function, or significant viral reactivation during the campaigns (Crucian 2009). Given the absence of the most likely causes of spaceflight-associated immune dysregulation (disrupted circadian rhythms, mission associated stress, isolation, etc.) bed rest most likely does not represent the best analog for exploration class spaceflight-associated immune dysregulation.

2. Ground-analog Animal Data

Ground analog animal data are essentially confined to hind limb elevation, suspension, unloading or restraint. Altered cytokine production patterns and reduced ability to fight infection have been observed using such analogs (Berry 1991, Sonnenfeld 1988). Although these results are extremely interesting, their direct correlation with human astronaut clinical risk from prolonged spaceflight-associated immune dysregulation is obviously debatable. There are obvious differences between humans and mice, and the animal suspension/restraint analogs are clearly different from prolonged spaceflight.

3. Ground-analog Cell Culture Data

Ground cell culture analogs for modeled microgravity such as clinorotation, bioreactor, High Aspect Ratio Vessel, all essentially subject cultured cells to a continuous free fall which has been shown to replicate some cellular effects of microgravity exposure. A variety of cellular effects, including altered actin polymerization, reduced lymphocyte locomotion, disrupted transmission of intracellular signals, and altered gene expression are all commonly observed that are believed to be similar to spaceflight observations (Pellis 1997, Licato 1999, Hughes Fulford 2005, Ward 2006). Sundaresan et al. identified the intracellular defect responsible for altered locomotion in modeled microgravity at the level of PKC or upstream, with lymphocyte calcium signaling pathways found to be functional (Sundaresan 2002). Hughes Fulford et al. found that alterations in 10 key genes were associated with simulated microgravity culture, indicating that the intracellular protein kinase A pathway was a key pathway altered during microgravity conditions and likely responsible for some of the observed spaceflight-associated immune dysregulation effects in humans (Hughes Fulford 2005). Ground cell-culture analogs may have significant utility for mechanistic studies that will determine the root causes of cell-specific *microgravity* induced immune system changes. However, any conclusions regarding clinical risk for exploration-class missions will require human subject studies, as variables such as stress and isolation cannot be replicated by these cellular analogs.

C. Microbial Environment and Virulence during Spaceflight

The incidence of infectious disease associated with early spaceflight missions was commonplace and observed on Apollo 7 (upper respiratory infection), Apollo 8 (preflight viral gastroenteritis), Apollo 9 (rhinitis and pharyngitis), and Apollo 13 (urinary tract infection). After Apollo 13, the implementation of the Health Stabilization Plan greatly decreased the frequency of reported disease **(**Billica1994) though its continued occurrence is well documented (see Table 13-1).

To minimize crew exposure to medically significant organisms, spacecraft air, surfaces, potable water, and food are evaluated prior to flight. In general, air, surface, and water samples reflect low concentrations of normal flora commonly seen in the environment (Castro, 2004, Pierson, 2001). *Staphylococcus*, *Micrococcus,* and *Bacillus* are the most common bacterial genera recovered from the air. The genera of bacteria most commonly cultured from spacecraft surfaces are *Staphylococcus*, *Micrococcus*, *Corynebacterium*, and *Bacillus*. *Aspergillus*, *Penicillium*, and *Cladosporium* are the most frequently cultured fungi from air or surfaces. Spacecraft potable water is maintained at very low microbial levels and contains common environmental microorganisms, such as *Burkholderia cepacia*, *Sphingomonas paucimobilis*, *Ralstonia, eutropha*, and *Methylobacterium* species (Castro, 2004; Pierson, 2001). The species associated with routine air, surface, and water monitoring generally do not contain medically significant organisms beyond opportunistic pathogens, such as *Staphylococcus aureus*. However, isolated incidences of contamination have been documented which suggest the potential of crew exposure to medically significant microorganisms during a mission (Ott, 2004). During the NASA Mir Program, an evaluation of multiple samples of water condensate from behind panels on the Mir Space Station indicated the presence of microorganisms not commonly isolated from routine environmental sampling, including *Escherichia coli*, *Serratia marcescens* and a *Legionella* species. In addition, microscopic analysis indicated the presence of protozoa, dust mites, and spirochetes (Ott, 2004). In addition to the environment, food is also monitored prior to flight. While the incidence of contamination is low, preflight analyses of food samples have indicated the presence of organisms such as *Salmonella typhimurium*, *Staphylococcus aureus*, *Enterobacter cloacae* and *Enterobacter* sakazakii (unpublished data). Contaminated lots are immediately removed before shipment for flight; however, these findings suggest a potential route of infection to the crew.

Determination of infectious disease risk is further complicated by a great deal of evidence that suggests genotypic and phenotypic changes, including virulence, occur in microorganisms in response to spaceflight (Nickerson, 2004). For over 40 years, multiple spaceflight experiments have indicated changes in phenotypic characteristics such as microbial growth, morphology, metabolism, genetic transfer, and antibiotic susceptibility (Nickerson, 2004). Recent ground-based analogs, using bioreactors designed to simulate aspects of the spaceflight environment, have supported the in-flight observations. These ground-based experiments also suggested changes in gene expression and an increase in virulence during flight (Nickerson, 2004). This evidence was supported by a flight experiment in 2006 aboard STS-115 in which *Salmonella typhimurium* was grown during flight and compared to identically cultured ground controls (Wilson, 2007). The cultures were either placed in an RNA fixative during flight or returned as

live cultures for virulence testing. The cultures grown aboard the Space Shuttle displayed an extracellular matrix that was not seen in the ground controls. Evaluation of the gene expression indicated 167 genes were differentially regulated compared to ground controls, with the conserved RNA-binding protein Hfq identified as a likely global regulator involved in the response to this environment. In addition, cultures grown during flight displayed a 2.7 fold lower LD50 in a murine model when compared to inoculation with ground control cultures (Wilson 2007).

In a follow up study designed to address potential mechanisms for such bacterial changes, the same authors have reported that spaceflight-induced increases in *Salmonella* virulence are regulated by media ion composition, and that phosphate ion is sufficient to alter related pathogenesis responses in a spaceflight analogue model (Wilson 2008). Using whole genome microarray and proteomic analyses from two independent Space Shuttle missions, they identified evolutionarily conserved molecular pathways in *Salmonella* that respond to spaceflight under all media compositions tested.

These data are suggestive that the microbial environment may be altered during spaceflight, with a potential for increased virulence. For exploration-class spaceflight, in conjunction with dysregulated immunity, this may represent an additional health risk to crewmembers.

V. Computer-based Simulation Information

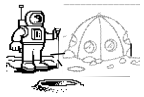
We know of no clinically relevant computer-based simulations for the human immune system.

VI. Risk in Context of Exploration Mission Operational Scenarios

The likelihood of an adverse clinical event (allergy, hypersensitivity, infection, malignancy) related to immunology is difficult to calculate due to the paucity of in-flight data and lack of understanding of the in-flight condition. Even when sufficient data from low Earth orbit missions of any duration are obtained, the situations experienced by crewmembers on such missions will likely be vastly different from the ones experienced by crewmembers on lunar long-duration or Mars missions. Current prediction of clinical risk in the context of different operational scenarios (vehicle, destination, duration, etc.) is best summarized as presented in Figure 13-6.

In general, the Discipline Team feels that low-Earth orbital flight of up to 6 months duration does NOT pose a significant health risk related to immunology. This is because of several factors unique to orbital flight. These include protection from certain kinds of deep-space high-energy radiation only beyond the Van Allen belt, as well as a readily-available return option. Also, orbital flight is likely a vastly different experience with regard to physiological stress than will be encountered during exploration class flight. It is expected that exploration class flight, with up to six-fold increases in mission duration, planetary exploration, and exposure to higher energy radiation, will increase the clinical risk. Radiation is a factor in assessing clinical risk related to immunology, due to the direct link between immunity, radiation, and cancer. Immune precursor cells residing in the marrow are particularly sensitive to radiation. Also, *if* immune dysregulation is found to persist during longer missions, the clinical risk related to tumor surveillance and development of malignancies may become significant.




	LEO-short duration	LEO-long duration	Lunar-short duration	Lunar-long duration	Mars-long duration
Vehicle	Shuttle	ISS	CEV/LSAM	CEV/LSAM+Outpost	CEV+MTV
Immune dysfunction	Yes	Yes	Yes	Yes	Yes
High-energy radiation	No	No	Yes	Yes	Yes
Return option	Yes	Yes	Limited	Limited	No
Medical capability	Limited	Limited	Limited	Limited	Limited
Clinical risk	**Low**	**Low**	**+/-**	**Potential elevated**	**Potential elevated**
Countermeasures	**No**	**No**		**Required**	**Required**

Figure 13-6. Perceived clinical risk related to spaceflight and immune dysregulation, in the context of mission duration, radiation environment, return option and predicted clinical capabilities.

VII. Conclusion

Determining the effect of space travel on the human immune system has proven to be extremely challenging. Limited opportunities for in-flight studies, varying mission durations, technical and logistical obstacles, small subject numbers, and a broad range of potential assays have contributed to this problem. Also, the inherent complexity of the immune system, with its vast array of cell populations, sub-populations, diverse regulatory molecules and broad interactions with other physiological systems makes determining precise variables to measure extremely complicated. There is also the challenge to determine the clinical significance of any observed immune alteration. Will such a change lead to disease, or is it a transient subclinical observation related to short-term stress? The effect of this problem may be observed by scanning publications dealing with immunity and spaceflight, which began to appear during the 1970s. Although individually they are each valid studies, the comprehensive literature to date suffers from widely varying sampling methods and assay techniques, low subject counts, and sometimes a disparate focus on narrow aspects of immunity.

The most clinically relevant data are derived from the in-flight human studies, which have demonstrated altered cell mediated immunity and reactivation of latent herpes viruses. Much more data are available from postflight testing of humans, with clear evidence of altered cytokine production patterns, altered leukocyte distribution, continued latent viral reactivation and evidence of dramatically altered virus-specific immunity. It is unknown if postflight assessments relate to the in-flight condition or are a response to landing stress and readaptation. In-flight

culture of cells has clearly demonstrated that immune cells are gravity-sensitive and display altered functional characteristics. It is unknown if these data are related to in vivo immune cell function, or are an artifact of microgravity culture. Ground analog testing of humans and animals, and microgravity-analog cell culture have all demonstrated utility. However, in all cases it is not known with certainty if these data would reflect similar testing during space travel. Given their ready availability, ground analogs may be extremely useful for assay development and the evaluation of potential countermeasures.

In general, the evidence base suffers from widely disparate studies on small numbers of subjects that do not directly correlate well with each other or spaceflight itself. This results in significant knowledge 'gaps' that must be filled by future studies, to completely determine any clinical risk related to immunity for human exploration-class space missions. These gaps include a significant lack of in-flight data, particularly during long-duration space missions. The International Space Station represents an excellent science platform with which to address this knowledge gap. Other knowledge gaps include lack of a single validated ground analog for the phenomenon, and a lack of flight-compatible laboratory equipment capable of providing a monitoring of astronauts (for either clinical or research purposes).

However, enough significant data exists, as described in this manuscript, to warrant addressing this phenomenon during the utilization phase of the ISS. NASA has recognized that if spaceflight-associated immune dysregulation persists during exploration flights in conjunction with other dangers such as high-energy radiation, the result may be a significant clinical risk. This emphasizes the need for an integrated comprehensive approach to determining the effect of prolonged spaceflight, separated from transient launch and landing stresses, on human immunity. After such studies, the phenomenon will be understood, and hopefully a monitoring strategy will have been developed that could be used to monitor the effectiveness of countermeasures.

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IX. List of Acronyms

AWO	Antarctic winter-over
CD_	cluster of designation (numerical nomenclature for cell surface proteins)
CEV	Crew Exploration Vehicle
CMI	cell-mediated immunity
CMV	cytomegalovirus
DNA	deoxyribonucleic acid
DSO	Detailed Supplemental Objective
EBER	Epstein-Barr Encoded RNA
EBV	Epstein-Barr virus
ESA	European Space Agency
EVA	Extra Vehicular Activity
Hfq	Host Factor the replication of the Qβ phage RNA
HMP	Haughton-Mars Project
HTBR	head-down-tilt bed rest
IE/E	intermediate early/early (EBV genes)
IFNg	interferon-gamma
IL-_	interleukin-_
ISS	International Space Station
JSC	Johnson Space Center
L-	launch – (days before launch)
LEO	low Earth orbit
LD50	median Lethal Dose
LSAM	Lunar Surface Access Module
MHC	Major Histocompatibility Complex
mRNA	messenger ribonucleic acid
MTV	Mars Transfer Vehicle
NEEMO	NASA Extreme Environment Mission Operations
NK	natural killer
PCR	polymerase chain reaction
PMA	phorbol 12-myristate 13-acetate
R+	return + (days after landing)
RT-PCR	real-time polymerase chain reaction
SDBI	Short Duration Bioastronautics Initiative (Shuttle experiment)
SLS	Spacelab Life Sciences (Shuttle payload)
SMO	Supplemental Medical Objective (ISS experiment)
STS	Space Transportation System
Th1	T-helper type 1 lymphocyte
Th2	T-helper type 2 lymphocyte
TNF	tumor necrosis factor
VZV	varicella-zoster virus

APPENDIX 13-1: SELECTED/REPRESENTATIVE EVIDENCE ARTICLES BY CATEGORY

A. FLIGHT DATA

In-flight human data

Category	Author/ Publication	NASA Category of Evidence	Summary of Evidence
Latent viral reactivation	Mehta 2000 J Infectious Dis	2	Shuttle astronauts: latent CMV reactivated before and during space flight, correlates with stress hormone levels and Ab titers.
	Payne 1999 ASEM	2	Assessment of in-flight reactivation of EBV via salivary detection of EBV DNA by PCR; 11 sero-positive Shuttle astronauts. Highest level of reactivation was Pre-flight, in-flight levels similar to post-flight. Suggests highest stress is before mission.
	Pierson 2005 Brain Behav. Immun.	2	Assessment of in-flight reactivation of EBV via salivary detection of EBV DNA by *quanitative* PCR; 32 Shuttle astronauts. Although subject incidence of shedding is actually more pre-flight that in-flight, the mean copy number per ml was much higher in-flight (417) as compared to pre-flight (40) and post-flight (44).
	Stowe 2001 ASEM	2	In-flight assessment during STS-95 includes elderly astronaut. Viral reactivation occurred during flight, as well as increased DHEAS/cortisol ratio. Suggests hormone changes during flight influence CMI.
	Mehta 2004 J. Med. Virol.	2	Short duration shuttle flights, subclinical latent VZV reactivation observed during flight (salivary VZV DNA), not present in control subjects.
Altered cell mediated immunity	Cogoli 1993 Environ Med	2	In-flight CMI test altered, T cell responses to mitogens depressed in-flight, post-flight. Clinical significance is unclear.
	Gmunder 1994 ASEM	2	Long duration/Mir: In-flight and post-flight CMI skin test reduced in some crewmembers. In-flight DTH alterations potentially associated with heavy stress EVA schedule.

In-flight animal data

	Lesnyak 1996 J Appl Physiol	2	Rats were dissected during the Shuttle SLS-2 mission, and biosamples returned to Earth. Summary: T cell activity decreased in-flight, spleen NK cell function reduced in-flight and post-flight, bone marrow NK cells unaltered. In flight: IL-1, IL-2, TNF reduced; post-flight: IFN levels reduced.

In-flight cell culture data

Altered NK cell function	Buravkova 2004 J Grav Physiol	2	ISS culture experiment: NK cell target interaction unaltered during flight. Low activity for flight and ground. (ISS-8)
Altered cytokine production	Chapes 1994 Adv Space Res	2	Secretion of IL-1 and TNFa by cell line following LPS stimulation elevated during flight.
Altered activation	Cogoli 1997 (review) Grav Space Biol	2	Cytoskeletal involvement, Ras/Rap, PKC all altered during microgravity. Leads to altered T cell responses, lack of cell activation. Review of in-flight studies.
	Cogoli 1993 J Leuko Biol	2	In-flight: suspended T cells fail to activated, bead-bound T cells do activate. Suggests failure of monocytes to act as APC during microgravity.
	Pippia 1996 J Biotechnol	2	In-flight stimulation of human PBMC with or without exogenous IL-1/IL-2 to determine if monocyte IL-1 defect explains in-flight lymphocyte function loss. Exogenous cytokine did not prevent loss of activity measured as mitotic index.
	Hughes-Fulford 2001 J Grav Physiol	2	Osteoblasts cultured during space flight demonstrated alterations in gene expression. Immediate early growth genes showed diminished mRNA induction in microgravity and the osteoblasts were slower to enter the cell cycle. Thus, microgravity alone may be a significant factor in bone loss associated with flight.

	Hughes-Fulford 2003 Adv Sapce Res	2	Multiple studies have shown that changes in cytoskeleton and extracellular matrix are associated with space flight, as well as actin and microtubule modifications.
	Hashemi 1999 FASEB J.	2	Activation of human PBMC/T cells during spaceflight results in failure to progress through CD69/CD25 expression. Indicates inhibition of T cell proliferation response occurs during early activation intracellular signaling steps.
	Meehan 1987 Adv Exp Med Biol	2	T cell proliferation is blunted during short duration missions. Similar responses seen to terrestrial stress and hypoxia. In flight studies needed to determine contribution of microgravity to observed effects.

Post-flight human data

Latent viral reactivation	Stowe 2001 Psychosom Med	2	Increases in EBV VCA antibodies were observed immediately before and following space flight. EBV NA antibodies were decreased at L-10 and found to further decrease following flight, indicating reduced CTL killing of infected cells. Those astronauts displaying EBV reactivation also had increases in stress hormone levels.
	Stowe 2000 Neuroimmunomod	2	Shuttle astronauts: lytic EBV reactivation observed pre-, and post-flight by distinguishing EBV-VCA and EBV-EA antibody titers. Correlates with stress hormone alterations.
Altered cytokine production/ leuk. dist.	Crucian 2000 J Ifn Ck Res	2	Altered cytokine profiles and leukocyte distribution following short duration flight.
	Maine 1991 ASEM	2	Post flight study with 5 cosmonauts: Enhanced IL-2 production but reduced IL-2r expression at landing. No changes in IL-1 expression or peripheral blood bulk phenotype.
Altered NK cell function	Konstantinova 1995 Acta Astronaut	2	21 day space flight results in post-flight reductions in NK cell levels, NK cell target binding and NK cell cytotoxicity. Also, lymphocytes demonstrated reduced capacity to produce TNF at landing day.
	Meshkov 1995 Acta Astronaut	2	NK cell function altered in Cosmonauts following space flight.
	Mehta 2001 J Appl Physiol	2	Short duration Shuttle flights: NK cell number unaltered post-flight, but NK cell cytotoxicity reduced following flight.
Altered leukocyte dist./neutrophil function	Stowe 1999 J. Leuk. Biol.	2	Following short duration space flight, crew members displayed neutrophillia with increased neutrophil adhesion. At landing there were alterations in the expression of adhesion molecules.
Altered monocyte function	Kaur 2005 Brain Behav Immunity	2	Monocyte study, short-duration post-flight: monocyte number was unlatered, but monocyte capacity to engulf E. coli, oxidative burst and degranulation were all reduced following landing. N=25 crew members.
Altered granulocyte funct.	Kaur 2004 Brain, Behav & Imm	2	Short duration Shuttle flights: Neutrophil number increased post-flight, phagocytosis and oxidative burst lower following flights of > 9 days.
Altered neuroendocrine resp.	Stowe 2003 ASEM	2	Post flight Shuttle study tests the hypothesis that mission duration impacts neuroimmune responses. Data suggest that sympathetic nervous responses dominate following shorter flights, whereas longer flights are characterized by glucocorticoid mediated changes.

Post-flight animal data

Cytokine dysregulation/ T cell function	Gould 1987 ASEM	2	Splenocytes from rats flown on Shuttle mission SLS-3 for 1 week demonstrated reduced IFNg production but normal IL-3 production following CON-A stimulation.
	Grove 1995 Exp Cell Res	2	Splenocytes from rats flown on Shuttle mission SLS-57 demonstrated reduced IL-2 production using TCR independent mitogen, but normal using TCR dependent mitogen. Splenocytes demonstrated increased integrin expression, whereas LN expression was decreased. Thus microgravity may induce lymphocyte redistribution among organs, influences organ-specific activation potential.
	Miller 1995 J App Physiol	2	Splenocytes and thymocytes were recovered post-flight from rats flown on STS-54 and secreted significantly higher titers of IL-3 and IL-6 (thymocytes only). Thus spaceflight can enhance expression of certain cytokines.
	Nash 1992 J App Physiol	2	Study of inguinal lymph node lymphocytes from rats flown on COSMOS 2044 mission. Proliferation and mitogenic responses of lymphocytes (3H method) not significantly altered. Production of IL-2 not altered. Suggests tissue-specific microgravity alterations.
	Sonnenfeld 1996 J Ifn Ck Res	2	Postflight study of Rhesus monkies flown on Russian COSMOS satellite. Reduced expression of IL-1 and IL-2 were observed post-flight. Marrow cells d
	Sonnenfeld 1992 J. App. Phys.	2	Post flight assessment of rats flown on COSMOS 2044 satellite. Leukocyte distribution was altered post-flight, as compared to control rats.
	Rykova 1992 J Appl Physiol	2	Post flight assessment of rats flown on COSMOS 2044 satellite. NK cell function was altered post-flight. Antiorthostatic suspension did not effect cytotoxicity. Effect was dependent on type of target cell utilized for assessment.
	Sonnenfeld 1990 ASEM	2	Post flight assessment of rats flown on COSMOS 1887 satellite. Leukocyte distribution was altered post-flight, as compared to control rats.

B. GROUND DATA

Ground-analog human data

Arctic analog	Crucian 2007 BMC Immunol	2	Haughton Mars Project, Devon Island, Canadian Arctic with 10 field season participants. Altered T cell function, cytokine profiles during mission.
Sleep deprivation	Shearer 2001 Allergy Clin Immunol	2	To assess if sleep deprivation may explain some space flight observations. Plasma cytokines measured. Data reveal that sleep loss increases levels of plasma sTNFa RI and IL-6 (that connect nervous, endocrine and immune systems).
Bed rest analog	Schmitt 1996 J Ifn Ck Res	2	Six subjects, 4 weeks head down tilt (HDT), two subjects 113 days HDT. Significant decrease in IL-2 secretion by PHA stimulated T cells. Increased IL-1 production.
	Uchakin 2007 ASEM	2	28 day bed rest results in changes in peripheral leukocyte distribution, T cell functional responses, cytokine secretion patterns, and reactivation of latent EBV.
Antarctic analog	Shearer 2002 J Allerg Clin Immunol	2	Evaluation of IL-10/IL-1ra and IFNg (anti-inflammatory vs. pro-inflammatory) in 21 Antarctic winter-over participants. Data showed time dependent increase in IFNg during mission and decreases in IL-1ra/IL-10 as compared to control subjects.
	Tingate 1997 Immunol Cell Biol	2	Alterations in T cell function, depressed CMI responses and reduced T cell proliferative capacity all observed during Antarctic winter over. Also, monocytosis was observed, and changes in the production of inflammatory cytokines. Viral reactivation is also observed during winter-over.
	Mehta 2000 J. Med. Virol	2	EBV reactivation and decreased CMI in Antarctic winter over subjects.

Ground-based animal data

Rat suspension, MC unloading/restraint	Berry 1991 J Ifn Res	2	Musculoskeletal unloading affected IFNg responses, while IL-1 and IL-2 were affectd by the physiological stress of restraint.
Mice, anti-orthostatic intolerance	Sonnenfeld 1988 Acta Microbiol Hung	2	Suspension model simulates some effects of microgravity. During suspension, secretion of interferon alpha, beta was inhibited, and mice showed a loss of resistance to infection (encephalomycarditis virus).

Ground-based cell culture data

	Licato 1999 Immunopharm	2	NK and LAK activity from PBMC stimulated during clinorotation is unaltered except for CD25 expression (IL-2r alpha chain). Ability of IL-2 to induce secondary cytokines completely abrogated.
	Schwarzenberg 1999 Adv Space Res.	2	Discussion of effect of microgravity on T cell activation. Effect is attributed to cytoskeletal changes and loss of IL-2 receptor. For ground assessments data from random-positioning machine are in good agreement with data from space flight.
	Boonyaratanakornkit 2005 FASEB J.	2	Ground based assessment of multiple gene expression during freefall culture in random-positioning machine. Alterations in expression of 10 key genes during simulated microgravity was identified. Data suggests that PKA pathway is key pathway related to loss of T cell activation during microgravity.

C. REVIEW ARTICLES

	Borchers 2002 Nutrition	n/a	Comprehensive review of spaceflight and immunity.
	Sonnenfeld 2002 Nutrition	n/a	Comprehensive review of spaceflight and immunity.
	Sonnenfeld 2002 (Med/Ex)	n/a	Comprehensive review of spaceflight and immunity.
	Sonnenfeld 1994 Acta Astronaut	n/a	Review of effect of space flight on cytokine production.
	Konstantinova 1993 J Leuko Biol	n/a	Review of Russian in-flight and post-flight immune data during long-duration flight. Summary: some alterations in Ig classes, lower in-flight DTH in 1 of 3 cosmonauts.
	Lesnyak 1993 J Leuko Biol	n/a	Review of data from rats flown during Space Shuttle mission. In-flight immune dysregulation is detailed.
	Taylor 1999 Adv Sp Biol Med	n/a	Review of immune changes during and after space flight.