The immune system in space and microgravity

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ABSTRACT


Space flight and models that created conditions similar to those that occur during space flight have been shown to affect a variety of immunological responses. These have primarily been cell-mediated immune responses including leukocyte proliferation, cytokine production, and leukocyte subset distribution. The mechanisms and biomedical consequences of these changes remain to be established. Among the possible causes of space flight-induced alterations in immune responses are exposure to microgravity, exposure to stress, exposure to radiation, and many more as yet undetermined causes. This review chronicles the known effects of space flight on the immune system and explores the possible role of stress in contributing to these changes. Key Words: EXTRATERRESTRIAL CONDITIONS, RESISTANCE TO INFECTION, STRESS

The ability to visit space for longer periods of time has opened up new aspects of research to determine the effects of space flight on numerous physiological functions. Among those being explored is the effects of space flight on the immune system (71). The purpose of this review will be to explore the history of the study of the effects of space flight on immunity. Because more opportunities have existed to determine the effects of space flight on immune responses of animals, the main focus of the review will be on animal models. Finally, a newly emerging area of the interaction of the immune system with the neuroendocrine system during space flight will be explored.

SPACE FLIGHT AND IMMUNITY

One of the important regulatory biology interactions affected by space flight is the regulation of the immune response. Alterations in the regulation of immunity could have profound effects on the ability of the host to resist infection and tumors. Reports on the effects of space flight on the immune system have been interesting but not definitive (17,40,58,65,77–80). These effects were transient and returned to normal after a postflight recovery period (17,40). Data obtained from human studies showed that there was inhibition of mitogen-induced blastogenesis of lymphocytes obtained from astronauts and cosmonauts after the Apollo-Soyuz test project, Skylab, Space Shuttle, and various Soviet flights (29,77–80). In these cases, the lymphocytes were obtained from the crew after their return flight. Samples taken from the crews as late as 7 d postflight indicated that the blastogenic response had not returned to normal. The defects in immune function may have been due to inhibition of macrophage function, but this has not been confirmed (78–80).

Soviet/Hungarian studies have also indicated that prolonged space flight resulted in hypoplasia of lymphoid organs and alterations in mitogen-induced blastogenesis (17,40). These effects were transient and returned to normal after a postflight recovery period (17,40). Data obtained from human studies showed that there was inhibition of mitogen-induced blastogenesis of lymphocytes obtained from astronauts and cosmonauts after the Apollo-Soyuz test project, Skylab, Space Shuttle, and various Soviet flights (29,77–80). In these cases, the lymphocytes were obtained from the crew after their return flight. Samples taken from the crews as late as 7 d postflight indicated that the blastogenic response had not returned to normal. The defects in immune function may have been due to inhibition of macrophage function, but this has not been confirmed (78–80).

Soviet/Hungarian studies have also indicated that some cosmonauts had a severe decrease in the ability of their leukocytes to produce interferon-α/β (an important cytokine that is both antiviral and immunoregulatory) when their blood was sampled and tested immediately after return from flight (75). Fifteen of 29 Apollo crew members developed bacterial or viral infections during their missions or immediately after return from flight (75). Fifteen of 29 Apollo crew members developed bacterial or viral infections during their missions or immediately after return from flight (24). The infectious agents included influenza viruses, Pseudomonas aeruginosa, and β-hemolytic streptococci (24). In the Apollo 13 mission, one crew member developed a urinary tract infection with P. aeruginosa during the flight (76). It is possible that the changes in immune responses...
induced by space flight (changes in cytokine production capacity in particular) could have contributed to decreased resistance to infection.

Human studies have indicated space-flight–induced changes in human cell culture activities such as a leukocyte blastogenesis (31–33) and signal transduction in leukocytes (34,56). Studies have also indicated that space flight alters leukocyte distribution (41,42,74), interferon and other cytokine production (16), and natural killer cell activity in humans when measured after flight. Interferon-α/β production was found to be dramatically enhanced when cultures of leukocytes were flown and stimulated in space (75). Interestingly, these were the cells from the same cosmonauts who were tested upon return to earth and shown to have inhibited interferon production (75). When assessed during flight, delayed hypersensitivity responses to common recall antigens are inhibited during both short-term (81) and long-term (20) space flights. Models of space flight such as isolation and long-term bed rest with head-down tilt have resulted in alterations of cytokine production and leukocyte function (55,57,67). As demonstrated with the interferon, some of these results have shown opposing results in different flight experiments, i.e., in one experiment an immune parameter is decreased, and in another it is unaltered or increased. This is probably due to specific experimental conditions, in vivo vs. in vitro experiments, species of experimental subject, and complicating additional variables that occur. Nevertheless, it is clear that exposure to space-flight conditions dramatically affects the immune system. A recent study has shown that exposure of humans to sleep deprivation, which can occur during space flight, results in alterations of TNF-α receptors and interleukin-6 levels in serum (61).

Studies involving antibody responses have been much more limited. Short-term space flight has resulted in no change in total immunoglobulin levels (82), whereas long-term space flight has shown a small increase in total immunoglobulin levels (29). Interestingly, a recent study has shown no effect of exposure on an Antarctic winter-over model of isolation in the ability of human subjects to mount an antibody response to bacteriophage ϕX-174 (60). Therefore, antibody responses may not be as sensitive to space flight or models of some components of space-flight conditions as are cell-mediated immune responses.

As a result of the limited opportunities for space flight, animal models have been used to study changes in immune responses. Immune responses of rodents have been the main subject of the studies. Cultures of rodent cells flown in space have shown altered cytokine production (7) and macrophage hematopoiesis and function (2). Chapes and associates (8) found enhanced secretion of several cytokines, including interferons-β and -γ, interleukin-1, and tumor necrosis factor-α, after murine cells were cultured and challenged to produce the cytokines during space flight. This is in accordance with results of Talas and associates (75), who found similar results using human cells. However, the ability of the tumor necrosis factor to kill cells was inhibited during space flight (83). This again suggests that some of the contradictory results on the effects of space flight on the immune system are due to the experimental conditions and parameters observed.

Mice were maintained in a “space cabin” environment in which barometric pressure was altered (19). These confined mice were more susceptible to mengovirus infection than were controls. These data suggested that maintenance of mice in this restricted environment with alterations in pressure could have resulted in alterations in immune responses that decreased their resistance to viral infections. By far, the most widely used model to simulate some aspects of weightlessness that occurs during space flight has been antorthostatic (15–20° head-down tilt), hypokinetic, hypodynamic suspension (no load on the hind limbs) (AOH). AOH suspension has been carried out either by tail (26,46) or by harness (47), but immunological results have been similar using both forms of suspension. The suspension model allows the development of physiological changes in muscle, bone, fluid shifts, and other parameters that are similar to some changes observed after weightlessness during space flight (46,47). Rats suspended in this model have shown involution of the thymus similar to that seen after space flight (17,73). No effect of suspension of rats on levels of immunoglobulin classes was observed (6), which is similar to the lack of effect of space flight on levels of circulating immunoglobulin classes of astronauts (82). Therefore, despite the obvious limitations of the model for simulating some of the effects of weightlessness seen during space flight, it has been useful in aiding the determination of which immunological parameters should be studied in rare flight experiments.

In our laboratory, we began our studies on the effects of space flight on immune responses by using the AOH suspension model. We utilized both the rat tail and harness suspension systems, and also developed a new mouse harness suspension model (72). Suspension of mice in the new model resulted in physiological changes that were similar to those observed using the rat suspension model (72). The first series of experiments we carried out involved the study of cytokines. Cytokines are soluble, non-antibody mediators that play a major role in cell-to-cell communication and regulation of immune responses (5). The first group of cytokines we studied was the interferons. Interferons are a family of proteins that have antiviral activity and several other activities, including regulation of immune responses (5). Interferon-α is produced primarily by leukocytes after stimulation with viruses, double-stranded RNA, or other nontoxic inducers (5). Interferon-β is produced primarily by fibroblasts by the same stimuli as interferon-α (5). In many cases, they are difficult to separate and are referred to commonly as interferon-α/β. The third type of interferon is interferon-γ. Interferon-γ is a product of an immune response by T-lymphocytes stimulated either with specific antigen or with a mitogen such as concanavalin-A or by natural killer cells (5).

The next studies performed by our laboratory showed that suspension of rats in an AOH model resulted in severe inhibition of interferon-α/β production (63). The rats were
suspended antiorthostatically in this tail suspension model for 2 wk and then challenged intravenously with polyriboinosinic-polyrribocytidylic acid (poly-I:C, a double-stranded RNA inducer of interferon-α/β. There was an 80% decrease in interferon-α/β production compared with normally caged controls (63). In more recent studies, rats were suspended antiorthostatically for 1–2 wk, and spleens were removed immediately after the rats were taken down from suspension. These results suggest that antiorthostatic suspension of rats resulted in altered interferon production, a finding similar to that observed when cosmonauts were tested for interferon production after space flight (75).

Similar results were observed when mice were suspended antiorthostatically (53). Using the mouse model, we were able to add a control for orthostatic suspension. In this control for the stress of confinement and stress of suspension, mice were suspended in a harness with no head-down tilt. Mice suspended for 1–2 wk in the antiorthostatic orientation showed severely inhibited interferon-α/β production compared with normally housed controls (53). Mice suspended in the orthostatic orientation showed no change in interferon-α/β production compared with controls (53). This suggested that the antiorthostatic orientation of suspension was required for inhibited interferon production, i.e., the stress of suspension alone could not account entirely for inhibited interferon production. Mice suspended antiorthostatically and then allowed to recover in normal caging for 1 wk regained their capacity to produce interferon-α/β (53). A more recent study carried out in our laboratory has also indicated that interferon-γ production can be altered by antiorthostatic suspension (4).

An additional suspension study using mice was carried out by Fleming and associates (18). In this study, they showed that mice suspended antiorthostatically had impaired ability to produce superoxide, decreased ability to kill phagocytosed bacteria (Propionibacterium acnes), and altered corticosterone levels that did not correlate with immunosuppressive effects of suspension (18). This indicated that antiorthostatic suspension could alter the inflammatory and phagocytic responses of the host. These data were not substantiated by others, who demonstrated no effect in a rat suspension model (44). The apparently contradictory data regarding suspension effects on inflammatory responses and phagocytosis raises a theme that was previously described regarding space-flight experiments. Sometimes, results appear to be contradictory. This is probably due to specific experimental conditions and complicating additional variables that occur.

In addition, we used the model to determine whether suspension resulted in alterations to resistance to infection. Female Swiss/Webster mice were inoculated with the D variant of encephalomyocarditis virus (EMC-D virus). EMC-D virus is a convenient virus to utilize, because alteration in a glucose tolerance test is all that is necessary to show successful infection with the virus. Females of the Swiss/Webster strain of mouse are normally resistant totally to infection with EMC-D virus (22). Resistance to EMC-D virus is mediated, at least in part, by interferon (22). Anti-orthostatically suspended mice became susceptible to infection, whereas orthostatically suspended mice remained resistant (22). Alterations in resistance to EMC-D virus correlated with alterations in interferon production. Therefore, antiorthostatic suspension-induced changes in interferon production could have contributed to the compromised resistance to EMC-D virus infection. This raises the possibility that changes in the immune systems that occur during or after space flight could contribute to possible compromised resistance to infectious diseases. These results indicated that changes in immunological parameters induced by antiorthostatic suspension had the potential to alter resistance to infection.

In view of the above findings, we had the opportunity to plan our first flight studies. Several rats were flown in SpaceLab-3, and experiments were carried out to determine the effects of flight on cytokine production (21). The rats were flown in the Space Shuttle for 7 d, and after landing, a transcontinental airplane flight and a 12-h delay occurred before sacrifice. This delay and flight could have affected the results we obtained. After sacrifice, spleens were removed from the rats and placed in individual cell culture. The cultures were then challenged with concanavalin-A to induce interferon-γ. After the appropriate time interval, the cultures were harvested and assayed for interferon-γ activity. Interferon-γ production was reduced significantly in cells taken from rats immediately after flight, compared with cells from control rats (21). This flight experiment yielded a result similar to that observed after antiorthostatic suspension of rats and consistent with the impaired interferon production by cosmonauts after flight (75).

Production of another cytokine, interleukin-3, was also measured in the same experiment on SpaceLab-3 used for production of interferon-γ (21). Interleukin-3 plays a major role as a growth factor for immunologically important cells. In this case, cells from rats flown in SpaceLab-3 showed the same pattern of production of interleukin-3 as did cells from control rats (21). These data suggested that not all immunological parameters are affected equally by space flight.

We were also fortunate to be able to participate in the Cosmos #1887 Soviet space flight (64). In this case, we extended our studies to other immunological areas. We carried out experiments to determine the effects of space flight on the distribution of leukocyte subpopulations. The distribution of leukocyte subpopulations has been shown to be an important indicator of normal immunological function, e.g., patients with acquired immune deficiency syndrome (AIDS) have an altered ratio of CD-4+ T lymphocytes to CD-8+ T-lymphocytes (31). In our first set of experiments, spleens were removed from 5 rats flown for 12.5 d on biosputnik Cosmos #1887. The biosputnik landed off course, and a 48-h delay and a transcontinental airplane flight occurred between landing and sacrifice of the rats. This delay could have affected the results we observed. The spleens were dissociated into individual cells, and separate samples were stained with different antibodies directed against markers on the surface of rat leukocyte populations. These antibodies were antiasialo GM-1 (natural killer cells),
OX-39 (interleukin-2 receptor), OX-1 (pan-leukocyte marker), W3/25 (CD-4+ T lymphocyte), OX-8 (CD-8+ T-lymphocyte), W3/13 (pan T-lymphocyte), OX-4 (polymorphic la-class II histocompatibility antigen), antirat IgG Fab’, antirabbbt IgG (irrelevant antibody control), and no antibody (negative control). The stained cells were then analyzed using a flow cytometer. Although there may be problems with nonspecific staining in these results, a trend suggesting a dramatic shift in the following cell populations compared with nonspecific staining in these results, a trend suggesting a shift in the following cell populations compared with synchronous (rats treated the same way as flight rats except for actual space flight) and ground controls: T-lymphocytes, CD-8+ T-lymphocytes, and interleukin-2 receptor-bearing T-lymphocytes (64). The increase in interleukin-2 receptor-bearing T-lymphocytes could have indicated an increased aberrant immunologic activity induced by space flight. However, this increased activity could have been held in check by the increased level of CD8+ T-lymphocytes. This altered ratio of T-lymphocyte subsets could also account for, at least in part, inhibited blastogenic responses and interferon production reported previously after space flight and may be related to an altered TH-1/TH-2 cytokine profile.

Additional studies carried out on rats flown in Cosmos #1887 involved bone marrow cells. Due to the limited number of bone marrow cells from the femur made available to us, only two cell populations were examined in the bone marrow, i.e., the total leukocyte population and the leukocytes carrying surface immunoglobulin. The analysis showed a large number of myelogenous cells bearing surface immunoglobulin from flown rats as compared with synchronous and ground control rats (64). Myelogenous cells are monocyte/macrophage precursors and would not have been expected to have a surface immunoglobulin marker. In addition, the bone marrow cells were also tested for their ability to respond to macrophage colony stimulating factor (M-CSF). M-CSF stimulates the division of monocyte-macrophage precursors (59). The bone marrow cells from flown rats were inhibited in their ability to form colonies in the presence of M-CSF, indicating a lack of division on the part of those precursor cells (64). The data with the bone marrow cells suggested that an unusual response for myelogenous cells to divide while they were still in the bone marrow of flown rats was induced by space flight and that those cells were refractory to additional exogenous stimulation by M-CSF. Monocytes/macrophages, therefore, are immunologically important cells that appear to be affected by space flight.

We were able to repeat the experiments described above for the Cosmos #1887 flight (64) during the Cosmos #2044 flight in 1990. Analysis of the data suggests a similar pattern of results to those observed during the Cosmos #1887 flight for both CSF and leukocyte-phenotyping studies (66). Additional studies indicated that natural killer cell activity was also affected by space flight (54). We participated in an additional study carried out by A. Lesnyak, using rats that were euthanized and dissected during a Space Shuttle flight (33). In this case, rats were euthanized 1 d before landing, and tissues were kept refrigerated until landing and analysis.

Control animals were euthanized at the same time on the ground, and tissues were maintained under similar conditions for the same duration as the flight samples. All samples were analyzed at the same time. Results indicated that both leukocyte blastogenesis and natural killer cell activity were inhibited in samples obtained during flight compared with ground controls (33). This indicates that the actual in-flight conditions contributed to the effects of space flight on the immunological parameters observed.

In another study, we carried out an experiment using pregnant rats flown on the Space Shuttle. Immune responses of dams, fetuses, and pups (fetuses and pups obtained after landing) were determined after landing (69). Interferon-γ production, leukocyte blastogenesis, and the response of immature cells to colony-stimulating factor all showed trends to inhibition in the dams but were unaffected in the pups and fetuses (when observation was possible) (69). These results suggest that the limited number of immune responses we observed in offspring of pregnant rats were not affected by space flight.

Recent studies have been carried out by Chapes and his associates (9,10) to determine whether there could be interventions that could prevent or ameliorate the effects of space flight on the immune system. In one set of experiments (10), treatment of rats before and during flight with interleukin-2 was utilized to determine whether detrimental effects of space flight on the immune system could be ameliorated. In this case, space-flight conditions induced very small changes in the immune system of control rats, so the effects of interleukin-2 were difficult to evaluate. In another experiment, rats were implanted with chambers that released insulin growth factor-1 and then subjected to space flight (9). The results suggested that insulin growth factor-1 could lessen some of the effects of space flight on the immune system but that space flight can also affect the normal response to the growth factor. Therefore, amelioration of space flight effects on immune responses remains an area that requires extensive future study.

Although most studies carried out in our laboratory and those of others on the effects of space flight on the immune system involved the use of rodents, there has been one limited study involving the use of rhesus monkeys. We were also able to participate in limited studies with monkeys. In a Russian Cosmos biosatellite mission, monkeys tested after return to earth showed decreases in interleukin-1 production, alterations in receptors for cytokines, and a decrease in the ability of bone marrow cells to respond to exogenous colony-stimulating factors (68). These results are similar to those observed in studies involving rodents and humans, and validate the use of the animal models for studying the effects of space flight on the immune system.

In view of the current limitations on space flight experiments caused by construction of the International Space Station, we have again focused on AOH suspension as a potential model for the effects of space flight on immune responses and resistance to infection. During the Cosmos 2044 flight, a parallel antioorthostatic suspension study was carried out. In this case, antioorthostatic suspension of rats
resulted in similar results to those seen after space flight with regard to the inhibited response of bone marrow cells to CSF but showed no correlation to the effects of space flight on subpopulations of leukocytes (66).

Other groups have also carried out studies to determine the effects of space flight and antithorostatic suspension on immune responses. These results are, in general, similar to our own results on leukocyte subset distribution and compartmentalization of the effects of space flight on immune responses (1,15,23,25,48–50). Additionally, an ability of the antithorostatic suspension to model effects of space flight on dynamic functional immune responses such as cytokine production but not on nondynamic immune parameters such as leukocyte subset distribution has been confirmed (1,4,15,23,25,48–50). Pecaut et al. (52) recently demonstrated that rats flown in space had changes in leukocyte subset distribution and that ground-based modeling did not show similar results. Therefore, despite its limitations, the antithorostatic suspension model is a very useful model for studying the effects of recreating some of the conditions occurring during space flight on immune responses and resistance to infection.

To take additional advantage of this model, because it has not been possible, to date, to carry out studies on the effects of space flight on resistance to infection, we have carried out ground-based studies to determine the effects of AOH suspension on resistance of mice to bacterial infection with Listeria monocytogenes (43,45). We have been able to show that mice subjected to AOH suspension have enhanced resistance to primary infection with L. monocytogenes (43,45). This was probably due to enhanced macrophage function and cytokine production in eliminating the pathogen (43,45). However, at the same time that resistance to primary infection was enhanced, the ability to generate long-term immunologic memory to the challenge organism was decreased after 7 d of suspension (43,45). Therefore, although initial resistance was enhanced by AOH suspension, the ability to develop long-term resistance to additional challenge with infectious organisms was compromised (43,45).

THE NEUROENDORINE SYSTEM, THE IMMUNE SYSTEM, AND SPACE FLIGHT

It is well-established that exposure to stress can result in alterations in immune responses and resistance to infection mediated by interaction of stress hormones from the sympathetic nervous system and the HPA axis with immune responses (62). Stress hormones, such as corticosteroids and catecholamines, have been shown to interact directly with cells of the immune system and to influence development of immune responses and the outcome of infections (62). Although such interactions of stress hormones and the immune system occur, they cannot explain all effects of the stress of space flight or AOH suspension on the immune system and resistance to infection (62).

Recently, we have also begun to develop a new approach to study the effects of the stress of space flight and AOH suspension on resistance to infection. This approach involves the study of the effects of changes in levels of catecholamines, such as norepinephrine, on the invading pathogens. The possibility that an infectious organism may respond to neuroendocrine signals, especially those that are elaborated during periods of stress, should not seem that unlikely. Recent original work by Lyte and his associates (32,35–37) and others establishing this principle has demonstrated that the catecholamines can profoundly influence the in vitro growth of gram-negative bacteria. Norepinephrine, in particular, was shown to increase the growth of members of the Enterobacteriaceae and Pseudomonadaceae families by over four logs as compared with bacteria cultured in control media. In addition to changes in growth, norepinephrine increased the production of virulence-associated factors on a protein equivalent basis as compared with controls. Production of the Shiga-like toxins by Escherichia coli 0157:H7 was found to be increased nearly 100-fold in the presence of norepinephrine (38). Elaboration of the K99 pilus adhesin by the enterotoxigenic E. coli (ETEC) pathogen B44, which is responsible for attachment of the bacterium to the intestinal wall, was increased over 1300-fold (39). Culture of pathogenic bacteria in norepinephrine-containing medium has also led to the discovery by Lyte and associates (37) of on of the first autoinducers of growth in bacteria. Importantly, this effect of catecholamines on bacterial growth has been shown to be nonnutritional in nature (32,37).

Studies involving the use of α and β adrenergic agonists and antagonists, as well as the less physiologically active enantiomer of norepinephrine, (+)-norepinephrine, suggested that a non-α, non-β adrenergic receptor-mediated process may play a role in norepinephrine-induced growth of gram-negative bacteria (32).

In our laboratory, we have adapted this system to study additional pathogens (27). We have shown that catecholamines can enhance the growth of Aeromonas hydrophila, an opportunistic gram-negative bacterial pathogen (27). Norepinephrine was the most effective catecholamine in this system, but epinephrine and dopamine were also effective (27). We have also shown that a siderophore-type mechanism may be involved in the interaction of catecholamines with bacteria, but confirmation will require additional study (28).

Stress can certainly play a role in the effects of space flight and AOH suspension on immune responses (70). Stress would not be the only factor involved, as multiple factors such as radiation exposure, microgravity exposure, and indirect effects from other body systems such as the musculoskeletal system could also play a role; however, it will be important to isolate and identify the effects of stress on resistance to infection to allow for future development of specific countermeasures. Although limited evidence to date suggests that stress hormones such as corticosteroids may not play a major role in the effects of space flight or AOH suspension on immune responses (18,30,51), the situation is unclear with regard to catecholamines, but preliminary evidence does suggest some correlation with space flight.
induced effects on immune responses (11). The area has not been fully or carefully investigated (11), and it is possible that stress-induced catecholamines could play a role in any alterations in resistance to infection induced by space flight or AOH suspension. The effect could be on the host immune responses or directly on the infectious organisms. This area should be a potential fruitful subject for future investigation.

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