Prolonged Anti-Orthostatic Hind Limb Unloading and Murine Allergic Asthma

Tae Young Jang; Min-Jeong Heo; Ah-Yeoun Jung; Young Hyo Kim

BACKGROUND: Hind limb unloading (HU) is one of the ground-based models of simulated microgravity. As bacterial and viral infections could affect the immune system, the immunologic effect of HU should be studied in a specific-pathogen-free (SPF) laboratory. However, a review of the literature did not reveal any studies on the immunologic effects of prolonged HU in a murine model of allergic disease. Accordingly, the present study was undertaken to evaluate the effect of HU in a murine model of allergic asthma in a SPF laboratory.

METHODS: Twenty BALB/c mice were allocated equally to Group A (control group), Group B (HU group), Group C (allergic group), or Group D (allergic + HU group). Weight gains, serum total and ovalbumin (OVA)-specific IgE, titers of IL-1, IL-5, IL-10, and IFN-γ in bronchoalveolar lavage (BAL) fluid, and histopathologic findings of the lungs were compared.

RESULTS: After 2 wk of HU, Group D showed significantly more weight loss (-2.0 ± 0.2 g) than Group C (-1.1 ± 0.4 g). Groups B and D showed significant increases in serum OVA-specific IgE as compared with Groups A and C. Group D had significantly lower titers of IL-5 (Group C: 53.0 ± 15.2 pg · ml^{-1}, Group D: 21.9 ± 13.9 pg · ml^{-1}), IL-10 (Group C: 430.8 ± 138.3 pg · ml^{-1}, Group D: 217.6 ± 51.2 pg · ml^{-1}), and IFN-γ (Group C: 104.3 ± 37.5 pg · ml^{-1}, Group D: 36.7 ± 12.8 pg · ml^{-1}) in BAL fluid than Group C. Peri-bronchiolar and pulmonary infiltrations of inflammatory cells were significantly greater in Group D than in Group C.

CONCLUSIONS: Prolonged HU may cause significant weight loss and aggravate disease courses.

KEYWORDS: hind limb suspension, asthma, specific pathogen-free organisms, allergy and immunology.

Jang TY, Heo M-J, Jung A-Y, Kim YH. *Prolonged anti-orthostatic hind limb unloading and murine allergic asthma.* Aerosp Med Hum Perform. 2015; 86(9):803–807.

Exposure to prolonged microgravity can cause significant immunologic dysregulation involving innate and specific immunity.[19] Although the exact pathophysiological mechanism of this immunologic dysregulation is unknown, increases in serum cortisol due to stress during spaceflights could be partially responsible.[16] Some studies have tried to evaluate the immunologic effect of microgravity on Earth and hind limb unloading (HU) or anti-orthostatic suspension is one of the most popular ground-based models of simulated microgravity.[7] HU causes a shift of body fluids to the central body area and disuse atrophy of posterior thigh muscles and, thus, simulates the musculoskeletal, cardiovascular, and immunologic changes observed during spaceflight.[15,17] Furthermore, from the immunologic perspective, prolonged HU can decrease delayed-type hypersensitivity and make organisms more susceptible to bacterial infections.[1]

Allergic asthma is a Th2-mediated chronic inflammatory disease of the lungs and airways. Although some studies have been performed on changes in humoral immunity after exposure to HU in normal, disease-free animals, no study had previously addressed the immunologic effects of prolonged simulated microgravity in a murine model of allergic disease. Therefore, we evaluated the effect of prolonged HU in a murine model of allergic asthma by evaluating: 1) weight gains; 2) serum total and ovalbumin (OVA)-specific IgE levels; 3) IL-1, IL-5, IL-10, and IFN-γ titers in bronchoalveolar lavage (BAL) fluid; and 4) lung parenchyma histopathologies.

From the Department of Otorhinolaryngology, Head and Neck Surgery, Inha University College of Medicine, Incheon, Republic of Korea.

This manuscript was received for review in January 2015. It was accepted for publication in June 2015.

Address correspondence to: Young Hyo Kim, M.D., Ph.D., Department of Otorhinolaryngology, Head and Neck Surgery, Inha University College of Medicine, 27 Inhang-ro, Junggu, Incheon 400-711, Republic of Korea; inhaorl@inha.ac.kr.

Reprint & Copyright © by the Aerospace Medical Association, Alexandria, VA.

DOI: 10.3357/AMHP.4265.2015

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-13 via free access

METHODS

Animals

Used were 20 female 8-10 wk old BALB/c mice (Orient Bio, Seongnam, Korea) free from murine specific pathogens. Animals were raised in a specific-pathogen-free (SPF) environment under a regular 12-h light-dark cycle and had free access to ovalbumin-free food and water. The experimental protocol used in this study was approved beforehand by the Animal Care and Use Committee of Inha University (INHA 130,404-202).

Equipment

In order to protect animals from possible contamination from the cages, potential air pollutants inside the cages must be completely isolated and, thus, we used Microisolator cages (Jeungdo Bio & Plant Co., Ltd, Seoul, Korea) which had stainless steel bars and contained a stainless steel water bottle holder to enable autoclave sterilization. To prevent necrosis of tails during HU, we sealed each tail with sponge and tape, and then fixed a plastic tube around it to prevent gnawing. In each case, the tail tip was suspended on a swivel-pulley, so that only the hind limbs were suspended; forelimbs touched the bottom of the cage, and the angle of suspension was adjusted to about 30°. A swivel pulley attached to a steel bar on each cage roof allowed mice to move freely in any direction using their forelimbs. However, they were unable to use their hind limbs on any structure.

Procedure

Allergic asthma was induced by administering OVA by intraperitoneal (I.P.) injection and intranasal (I.N.) challenge; we adopted the 35-d protocol widely used and previously verified with a slight modification.[9,12] Briefly, animals were systemically sensitized by injecting OVA (40 $\mu g \cdot kg^{-1}$, I.P., Sigma-Aldrich, St. Louis, MO) diluted in sterile saline and alum adjuvant (40 $mg \cdot kg^{-1}$) on experimental days 1, 5, 14, and 21. Throughout days 22 to 35 (inclusive), each mouse underwent an I.N. challenge with OVA diluted in sterile saline (20 μl of 25 $mg \cdot ml^{-1}$ OVA per mouse). Mice were sacrificed 24 h after the last I.N. challenge.

Mice were assigned to four groups ($N = 5$ per group). Group A animals (the control group) received I.P. injections and I.N. challenges of sterile saline on the experimental days mentioned above and were unrestrained, that is, they were able to move forelimbs and hind limbs freely. Group B (the HU group) were administered I.P. injections and I.N. challenges with sterile saline, as mentioned for Group A, and exposed to 2 wk of HU from experimental days 22 to 35 (inclusive; starting the day of the I.N. challenge). Group C (the allergic group) were exposed to OVA as described in the section above, but not exposed to HU, and Group D (the allergic + HU group) were exposed to the OVA challenge and 2 wk of HU.

Animal weights were measured before and after the 5-wk experimental period and mean weight gains in the four study groups were compared. Serum was collected using an aortic puncture technique and BAL fluid was harvested by performing lavage with sterile saline (3 ml) through an intratracheal polyethylene tube, as previously described.[10] After staining centrifuged preparations with Diff-Quik (Baxter Scientific, Miami, FL) according to the manufacturer's instructions, differential cell counts for eosinophils, neutrophils, and lymphocytes among 500 cells were performed at a magnification of 1000×.

Titers of total and OVA-specific IgE in serum were obtained by ELISA, as previously described.[11] A standard concentration curve obtained using a mouse IgE standard (BD Biosciences, San Diego, CA) was used to determine total IgE levels. For OVA-specific IgE, we measured optical densities at 450 nm and compared these values rather than using values obtained using a standard curve. Titers of IL-1β, IL-5, IL-10, and IFN-γ in BAL fluid were also obtained using appropriate ELISA kits (BioSource International, Camarillo, CA).

Lung tissues were fixed in a 10% formalin solution for 3 wk and embedded in paraffin. Sections (4 μm) were prepared and stained using hematoxylin and eosin to determine degrees of inflammatory cell infiltration. Numbers of infiltrating inflammatory cells around the bronchioles were counted in 20 random high-power (400×) fields per section, by two independent, impartial observers.

Statistical Analysis

Nonparametric tests, that is, the Kruskal–Wallis test and the Mann–Whitney *U*-test, were used to compare groups. All results are presented as mean ± SD. Statistical significance was accepted for *P*-values of < 0.05 and the analysis was performed using SPSS ver. 19.0 (IBM, Armonk, NY).

RESULTS

After the 35-d treatment period, no significant difference in weight gain was observed between Group A (control group: 1.3 ± 0.5 g) and Group B (HU group: 0.7 ± 1.3 g). However, significant weight loss was found in Group D (allergic + HU group, −2.0 ± 0.2 g) as compared with Group C (allergic group, −1.1 ± 0.4 g, $P = 0.029$) (**Fig. 1**).

Total IgE levels were similar in the nonallergic groups (Group A 0.5 ± 0.2 $\mu g \cdot ml^{-1}$, Group B 0.6 ± 0.6 $\mu g \cdot ml^{-1}$) and in the allergic groups (Group C 6.0 ± 0.8 $\mu g \cdot ml^{-1}$, Group D: 5.1 ± 2.4 $\mu g \cdot ml^{-1}$, **Fig. 2A**). However, groups exposed to HU (Groups B and D) showed significant increases in OVA-specific IgE levels as compared with groups not exposed (Groups A and C), respectively (**Fig. 2B**).

After exposure to prolonged HU, Group B had significantly elevated IL-β (Group A: 54.8 ± 25.1 $pg \cdot ml^{-1}$, Group B: 139.1 ± 98.3 $pg \cdot ml^{-1}$, $P = 0.047$) and IFN-γ (Group A: 29.3 ± 2.7 $pg \cdot ml^{-1}$, Group B: 38.3 ± 4.0 $pg \cdot ml^{-1}$, $P = 0.016$) titers in the BAL fluid. Group D had significantly lower titers of cytokines in the BAL fluid than Group C: IL-5 (Group C: 53.0 ± 15.2 $pg \cdot ml^{-1}$, Group D: 21.9 ± 13.9 $pg \cdot ml^{-1}$, $P = 0.029$); IL-10 (Group C: 430.8 ± 138.3 $pg \cdot ml^{-1}$, Group D: 217.6 ± 51.2 $pg \cdot ml^{-1}$, $P = 0.029$); and IFN-γ (Group C: 104.3 ± 37.5 $pg \cdot ml^{-1}$, Group D: 36.7 ± 12.8 $pg \cdot ml^{-1}$, $P = 0.029$) (**Fig. 3A**). Group D tended to have fewer eosinophils, fewer

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-13 via free access

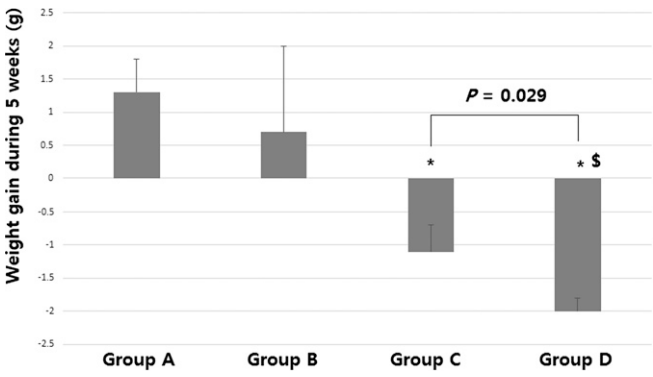

Fig. 1. Weight gain after the 5-wk experimental period. Group A: control group, Group B: hind limb unloading (HU) group, Group C: allergic group without HU exposure, Group D: allergic + 2 wk of HU group. Kruskal-Wallis and Mann-Whitney *U*-tests: *significantly different vs. Group A, $P < 0.05$; $^{\$}$significantly different vs. Group B, $P < 0.05$; error bar: standard deviation.

lymphocytes, and more neutrophils in the BAL fluid than Group C ($P \geq 0.05$) (**Fig. 3B**).

No infiltration of inflammatory cells around the bronchioles or into the lung parenchyme was observed in Groups A or B. However, peribronchiolar and pulmonary infiltrations by inflammatory cells were significantly greater in Group D than in Group C as determined visually (**Fig. 4A**) and by counting (**Fig. 4B**).

DISCUSSION

Immune dysregulation is one of the negative health effects caused by orbital spaceflight.[2,4,5] Although they are completely isolated from pathogens on Earth, astronauts report increased incidences of infectious diseases and hypersensitivity reactions.[14] Astronauts experience immune dysregulation not only after landing, but also during space missions and, frequently, these conditions persist for more than 6 mo during spaceflight.[3,8]

In their recent study, Mehta et al. suggested changes in cytokine levels were closely correlated with the increased prevalences of infectious diseases among crewmembers, and that a shift in cytokine profile from Th1 to Th2 might be the cause.[13] In fact, allergic asthma and rhinitis are associated with exacerbated Th2 immune response and, thus, a change in cytokine balance from Th1 to Th2 could affect infections and allergic disorders.

A review of the literature showed this is the first study to evaluate the effect of long-term simulated microgravity using a murine HU model on the clinical course of allergic asthma. With respect to weight gain, experimental animals without asthma, regardless of HU exposure, showed no significant difference in weight gain, which concurs with previous results,[7,21] and shows that when animals are provided with sufficient food, HU alone does not cause significant weight loss. However, asthmatic mice in Group D (allergic + HU group) showed significantly more weight loss than their non-HU counterparts (Group C, allergic group), which suggests that allergic asthma and prolonged HU exerted a synergistic cachectic effect.

Few studies have addressed the effect of prolonged HU on serum immunoglobulin levels. In the present study, prolonged HU did not induce any significant changes in serum IgE levels in normal experimental animals, but OVA-specific IgE levels were significantly increased by HU in both normal and asthmatic mice, and asthmatic animals showed greater increases in OVA-specific IgE levels after HU exposure. This result is consistent with previous findings; for example, in one study, prolonged HU exposure and stimulation with CpG significantly increased serum IgE levels, but not serum IgG or IgM levels.[20] Therefore, we suggest allergic diseases could be exacerbated by increased IgE levels caused by prolonged HU.

Some studies have been conducted on the effects of prolonged HU on the titers of cytokines in normal experimental animals. Felix et al. found that IL-1β titers in the serum, spleen, and lymph nodes were significantly elevated after prolonged HU.[6] Gaignier et al. reported IL-1β was not elevated in LPS-stimulated splenic lymphocytes of mice exposed to 3 wk of HU.[7] In the present study, IL-1β titers in BAL fluid were significantly increased after prolonged HU in normal mice, but in the asthmatic mice, no significant difference was found.

Little information is available on the effects of HU exposure on Th2 cytokine levels. In a previous study, IL-5 titers of splenic lymphocytes (obtained after 3 wk of HU exposure) and stimulated with LPS or ConA were relatively unchanged.[7] In the present study, IL-5 levels in BAL fluid were not significantly increased by exposure to HU in Group B (the HU group). However, IL-5 levels were significantly reduced by exposing allergic mice to HU. IL-5 is an eosinophilopoietic cytokine and, thus, reductions in its levels due to HU could in part explain reduced eosinophilic infiltration.

IL-10 is an immunomodulatory cytokine. In a previous study, exposure to HU up-regulated IL-10 levels nonsignificantly in peripheral bone marrow cells.[20] Gaignier et al. also reported no significant change in IL-10 titers

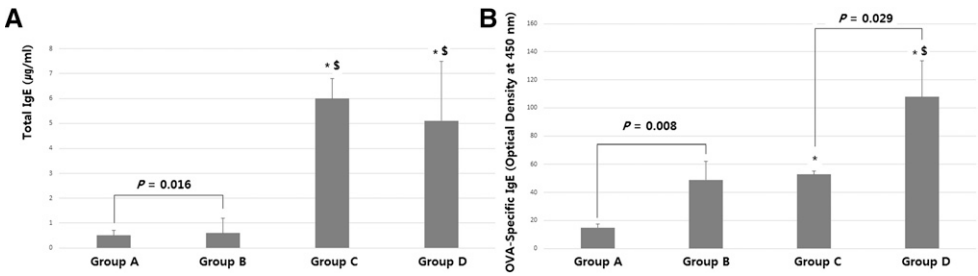

Fig. 2. A) Serum titers of total IgE and B) ovalbumin-specific IgE. Group A: control group, Group B: hind limb unloading (HU) group, Group C: allergic group without HU exposure, Group D: allergic + 2 wk of HU group. Kruskal-Wallis and Mann-Whitney *U*-tests: *significantly different vs. Group A, $P < 0.05$; $^{\$}$significantly different vs. Group B, $P < 0.05$; error bar: standard deviation.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-13 via free access

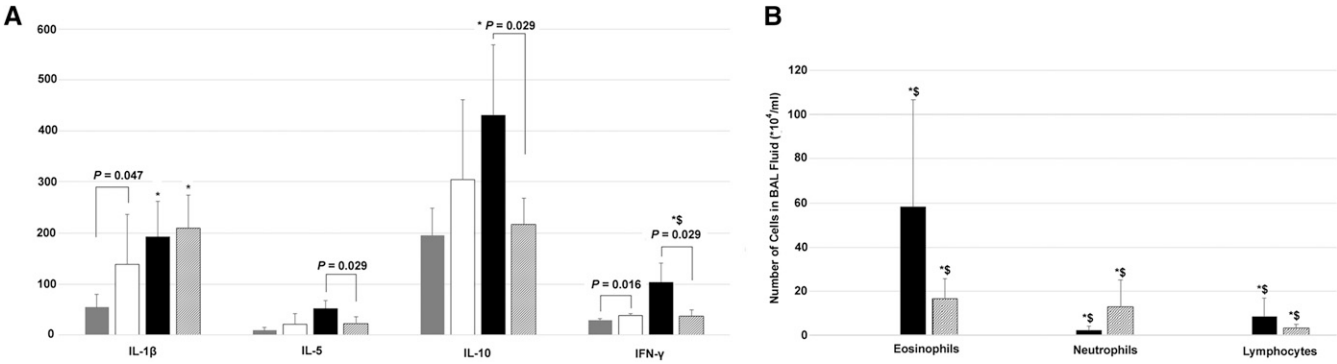

Fig. 3. A) Cytokine profiles in the bronchoalveolar lavage (BAL) fluid. B) Numbers of different inflammatory cells in the bronchoalveolar lavage fluid. Group A: control group (grey bars), Group B: hind limb unloading (HU; white bars) group, Group C: allergic group without HU exposure (black bars), Group D: allergic + 2 wk of HU group (striped bars). Kruskal-Wallis and Mann-Whitney *U*-tests: *significantly different vs. Group A, $P < 0.05$; $^{\$}$significantly different vs. Group B, $P < 0.05$; error bar: standard deviation.

in LPS-stimulated lymphocytes.[7] In the present study, no significant difference was observed between IL-10 levels in the BAL fluids of Groups A and B. However, in asthmatic animals, IL-10 levels were significantly reduced by exposure to HU. These findings suggest the possibility that some dysregulation of immune-modulatory function could occur in asthmatic individuals exposed long-term to microgravity.

The effect of simulated microgravity on IFN-γ levels remains controversial. In one study, IFN-γ showed a nonsignificant tendency to increase with LPS stimulation after HU exposure.[7] On the other hand, IFN-γ showed a nonsignificant decreasing tendency in ConA-stimulated lymphocytes.[7] In the sera of patients exposed to 45 d of head down bed rest, IFN-γ was found to decrease gradually, but no significant difference was observed until HU had been administered for 2 wk (maximum decrease was observed after 45 d of HU).[20] Felix et al. reported that after long-term HU exposure, serum IFN-γ levels were significantly reduced in serum, slightly reduced in the lymph nodes, but not significantly changed in spleen tissues.[6] In the present study, IFN-γ levels in BAL fluid were significantly increased by HU in Group B versus normal controls, but were significantly reduced by HU exposure in Group D as compared with Group C. Because IFN-γ plays important roles in innate and adaptive immune responses against bacterial and viral infections, these findings suggest asthmatic individuals are more vulnerable to exacerbation of auto-immune and/or auto-inflammatory responses induced by long-term exposure to microgravity.

In a previous study, prolonged exposure to HU reduced serum eosinophil and lymphocyte levels, but significantly increased neutrophil levels.[18] However, no previous study has addressed changes in inflammatory cells in BAL fluid after HU. We found no inflammatory cells in BAL fluid after exposing 'normal' animals to HU, but in asthmatic animals, prolonged HU exposure tended to reduce eosinophil and lymphocyte counts and to increase neutrophil counts in BAL fluid. Studies with larger populations are required to confirm these tendencies.

Several previous studies on HU and its immunologic effects failed to mention SPF status. In the present study, we devised a HU cage compatible with protocols required to maintain a SPF laboratory environment. During the entire 35-d experimental period, a pathogen-free environment was maintained without any contamination concerns and no animal in the HU group exhibited abnormal behaviors, such as leaning on walls or suspending itself from any structure, and, thus, we were able to minimize the risks of incidental air-borne bacterial and viral infections.

In nonasthmatic mice, the administration of HU for 2 wk had no notable effect on the histopathological findings of lung tissues. However, the administration of HU to asthmatic mice dramatically increased pulmonary infiltration by inflammatory cells. One of the pitfalls of our study is the small number of animals in each experimental group. Because only one mouse could be raised in one HU cage, we prepared 20 independent cages for the animals. In the near future, we hope that a larger-scale

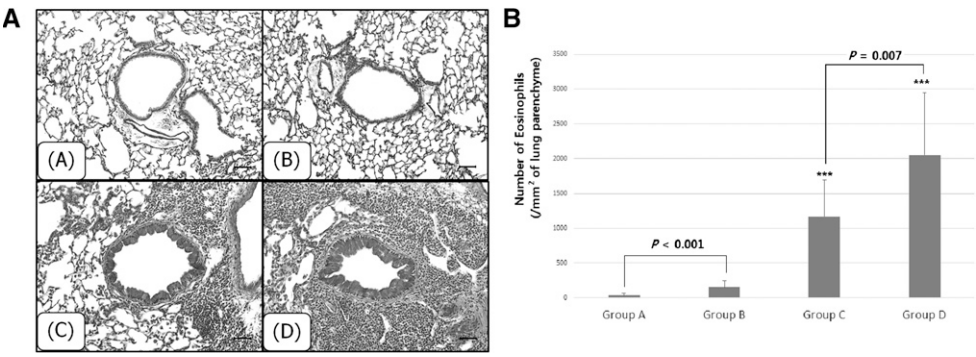

Fig. 4. A) Histopathologic findings of lung tissues. Group A: control group, Group B: hind limb unloading (HU) group, Group C: allergic group without HU exposure, Group D: allergic + 2 wk of HU group. Hematoxylin and eosin staining, ×200, scale bar = 50 μm. B) Numbers of infiltrating cells in 1 mm^2 of lung parenchyma. Kruskal-Wallis and Mann-Whitney *U*-tests: ***significantly different vs. Group A, $P < 0.001$.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-13 via free access

study on larger populations will yield more meaningful data. In terms of extrapolating our findings to health risks faced by astronauts participating in space missions, we believe allergic symptoms and Th2 responses are likely to be exacerbated. In future spaceflights, this possibility should be kept in mind and evaluated more thoroughly in astronauts. In conclusion, simulated microgravity by prolonged HU was found to cause significant weight loss and to aggravate the clinical course of allergic disease by: 1) increasing serum specific IgE levels; 2) changing cytokine levels in BAL fluid, such as IL-5, IL-10, and IFN-γ; and 3) increasing pulmonary infiltration by inflammatory cells in our murine model of allergic asthma.

ACKNOWLEDGMENTS

This study was supported by a grant (NRF-2013M1A3A3A02042309) from the Space Core Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT and Future Planning (MSIP), the Basic Science Research Program of the NRF funded by the Korean Ministry of Education, Science, and Technology (NRF-2013R1A1A1006382), and by Inha University Research Grants. The authors of this study appreciate the invaluable help afforded by Byeong Jun Cho, who made a substantial contribution in the development of the HU cage.

The authors have no potential conflict of interest to declare. This article was presented at the E-poster session at the 16th World Congress of Rhinology, April 30, 2015; Sao Paulo, Brazil.

Authors and affiliation: Tae Young Jang, M.D., Ph.D., Min-Jeong Heo, M.S., Ah-Yeoun Jung, M.S., and Young Hyo Kim, M.D., Ph.D., Department of Otorhinolaryngology, Head and Neck Surgery, Inha University College of Medicine, Incheon, Republic of Korea.

REFERENCES

1. Belay T, Aviles H, Vance M, Fountain K, Sonnenfeld G. Effects of the hindlimb-unloading model of spaceflight conditions on resistance of mice to infection with Klebsiella pneumoniae. J Allergy Clin Immunol. 2002; 110(2):262–268.
2. Blaber E, Marcal H, Burns BP. Bioastronautics: the influence of microgravity on astronaut health. Astrobiology. 2010; 10(5):463–473.
3. Crucian B, Stowe R, Mehta S, Uchakin P, Quiriarte H, et al. Immune system dysregulation occurs during short duration spaceflight on board the space shuttle. J Clin Immunol. 2013; 33(2):456–465.
4. Crucian B, Simpson RJ, Mehta S, Stowe R, Chouker A, et al. Terrestrial stress analogs for spaceflight associated immune system dysregulation. Brain Behav Immun. 2014; 39:23–32.
5. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. Neuroimmunomodulation. 2009; 16(5):300–317.
6. Felix K, Wise K, Manna S, Yamauchi K, Wilson BL, et al. Altered cytokine expression in tissues of mice subjected to simulated microgravity. Mol Cell Biochem. 2004; 266(1-2):79–85.
7. Gaignier F, Schenten V, De Carvalho Bittencourt M, Gauquelin-Koch G, Frippiat JP, Legrand-Frossi C. Three weeks of murine hindlimb unloading induces shifts from B to T and from th to tc splenic lymphocytes in absence of stress and differentially reduces cell-specific mitogenic responses. PLoS One. 2014; 9(3):e92664.
8. Guéguinou N, Huin-Schohn C, Bascove M, Bueb JL, Tschirhart E, et al. Could spaceflight-associated immune system weakening preclude the expansion of human presence beyond Earth's orbit? J Leukoc Biol. 2009; 86(5):1027–1038.
9. Jang TY, Park CS, Kim KS, Heo MJ, Kim YH. Benzaldehyde suppresses murine allergic asthma and rhinitis. Int Immunopharmacol. 2014; 22(2): 444–450.
10. Kim YH, Park CS, Lim DH, Ahn SH, Son BK, et al. Beneficial effect of anti-interleukin-33 on the murine model of allergic inflammation of the lower airway. J Asthma. 2012; 49(7):738–743.
11. Kim YH, Yang TY, Park CS, Ahn SH, Son BK, et al. Anti-IL-33 antibody has a therapeutic effect in a murine model of allergic rhinitis. Allergy. 2012; 67(2):183–190.
12. Kim YH, Park CS, Lim DH, Son BK, Kim JH, et al. Antiallergic effect of anti-siglec-F through reduction of eosinophilic inflammation in murine allergic rhinitis. Am J Rhinol Allergy. 2013; 27(3):187–191.
13. Mehta SK, Crucian BE, Stowe RP, Simpson RJ, Ott CM, et al. Reactivation of latent viruses is associated with increased plasma cytokines in astronauts. Cytokine. 2013; 61(1):205–209.
14. Mermel LA. Infection prevention and control during prolonged human space travel. Clin Infect Dis. 2013; 56(1):123–130.
15. Morey-Holton E, Globus RK, Kaplansky A, Durnova G. The hindlimb unloading rat model: literature overview, technique update and comparison with space flight data. Adv Space Biol Med. 2005; 10:7–40.
16. O'Donnell PM, Orshal JM, Sen D, Sonnenfeld G, Aviles HO. Effects of exposure of mice to hindlimb unloading on leukocyte subsets and sympathetic nervous system activity. Stress. 2009; 12(1):82–88.
17. Sonnenfeld G, Morey ER, Williams JA, Mandel AD. Effect of a simulated weightlessness model on the production of rat interferon. J Interferon Res. 1982; 2(4):467–470.
18. Taylor GR, Neale LS, Dardano JR. Immunological analyses of U.S. space shuttle crewmembers. Aviat Space Environ Med. 1986; 57(3):213–217.
19. Taylor GR, Janney RP. In vivo testing confirms a blunting of the human cell-mediated immune mechanism during space flight. J Leukoc Biol. 1992; 51(2):129–132.
20. Xu X, Tan C, Li P, Zhang S, Pang X, et al. Changes of cytokines during a spaceflight analog–a 45-day head-down bed rest. PLoS One. 2013; 8(10):e77401.
21. Yamauchi K, Hales NW, Robinson SM, Niehoff ML, Ramesh V, Pellis NR, et al. Dietary nucleotides prevent decrease in cellular immunity in ground-based microgravity analog. J Appl Physiol (1985). 2002; 93(1):161–166.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-13 via free access