

Effects of the hindlimb-unloading model of spaceflight conditions on resistance of mice to infection with *Klebsiella pneumoniae*

Tesfaye Belay, PhD, Hernan Aviles, MD, PhD, Monique Vance, BS,
Kimberly Fountain, BS, and Gerald Sonnenfeld, PhD Atlanta, Ga

Background: It has been well documented in several studies that many immunologic parameters are altered in experimental animals and human subjects who have flown in space. However, it is not fully known whether these immunologic changes could result in increased susceptibility to infection. Hindlimb (antiorthostatic) unloading of rodents has been used successfully to simulate some of the effects of spaceflight on physiologic systems.

Objective: The objective of this study was to determine the effect of hindlimb unloading on the outcome of *Klebsiella pneumoniae* infection in mice.

Methods: Hindlimb-unloaded, hindlimb-restrained, and control mice were intraperitoneally infected with one 50% lethal dose of *K pneumoniae* 2 days after suspension. Mortality and bacterial load in several organs were compared among the groups.

Results: Unloaded mice showed significantly increased mortality and reduced mean time to death compared with that seen in the control groups. Kinetics of bacterial growth with smaller infective doses revealed that control mice were able to clear bacteria from the organs after 30 hours. In contrast, unloaded mice had continued bacterial growth at the same time point.

Conclusion: The results of this study suggest that hindlimb unloading might enhance the dissemination of *K pneumoniae*, leading to increased mortality. The complex physiologic changes observed during hindlimb unloading, including stress, have a key role in the pathophysiology of this infection. (J Allergy Clin Immunol 2002;110:262-8.)

Key words: Hindlimb, unloading, antiorthostatic, suspension, stress, spaceflight, resistance, mice, infection

Research focusing on the influences of stress on the normal functioning of the immune system and the pathogenesis of infectious disease has received considerable

Abbreviations used

CFU: Colony-forming unit
LD₅₀: 50% lethal dose
TSA: Tryptic soy agar
TSB: Tryptic soy broth

attention in the past few years. Several studies have increased our understanding of the complex interactions between the central nervous system and the immune system regarding the role of stress in resistance to infection.¹⁻⁵ Included among those interactions are the linkages between the neuroendocrine system and immune system that occur, especially during spaceflight.^{2,6-10} It is well known that immune function is altered as a result of spaceflight.⁶⁻¹² The immunologic changes that occur after spaceflight include changes in immune cell functions (eg, cytokine production), changes in leukocyte distribution, reduction in proliferation of T lymphocytes after mitogen stimulation, and reduction in the total number of leukocytes.⁶⁻¹² Multiple factors that occur during spaceflight appear to play a role in the immunologic changes observed after spaceflight, including stress, exposure to microgravity, and exposure to radiation.⁶⁻¹² Alterations in the physiology of the host, particularly the immune system, could have profound effects on the ability of the host to resist infections. Although spaceflight exposure results in alterations in immune responses, the significance of those changes regarding susceptibility to infection remains to be established.

Because spaceflight experiments are costly and of limited availability, models have been developed to simulate some of the conditions that occur during spaceflight. For rodents, the most widely used model is hindlimb (antiorthostatic) unloading. Hindlimb unloading of rodents is a ground-based stress model that resembles some of the physiologic changes observed during spaceflight.^{11,13-16} In the model rodents are suspended with no load bearing on the hind limbs and with a head-down tilt of 15° to 20°.^{11,13-16} These conditions induce muscle and bone loss and a fluid shift to the head, which are similar to changes observed during spaceflight.^{13-15,17} Use of this model has shown functional changes in the immune system as well. These changes include thymic involution,¹⁶ decreased interferon production,¹⁸ and decreased bone marrow colony formation.¹⁴

From the Department of Microbiology, Biochemistry, and Immunology, Morehouse School of Medicine, Atlanta.

The National Aeronautics and Space Administration (NASA) supported this work through the NASA Cooperative Agreement NCC 9-58 with the National Space Biomedical Research Institute. Facilities used at Morehouse School of Medicine for these studies were supported by the National Institute of Health under the RCMI program award no. 5G12 RR/AL-03034.

Received for publication March 5, 2002; revised May 8, 2002; accepted for publication May 13, 2002.

Reprint requests: Gerald Sonnenfeld, PhD, Department of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, 720 Westview Drive SW, Atlanta, GA 30310-1495.

© 2002 Mosby, Inc. All rights reserved.

0091-6749/2002 \$35.00 + 0 1/84/126459

doi:10.1067/mai.2002.126459

Klebsiella pneumoniae is a gram-negative bacteria that is a major pathogen in individuals exposed to stress and who are immunosuppressed as a result of the stress exposure, including conditions such as major trauma and surgery.¹⁹ *K pneumoniae* has played a major role in the development of sepsis in patients who have experienced severe trauma, possibly because of translocation of organisms from the gut to the circulation.¹⁹ Therefore it has the potential of becoming a pathogen for individuals exposed to spaceflight conditions. The present study was carried out to determine the effects of exposure of mice to spaceflight-like conditions of the hindlimb-unloading model on resistance to infection with *K pneumoniae*. Additional studies on the possible mechanisms involved in hindlimb unloading-induced changes in resistance to infection were carried out.

METHODS

Bacteria

A clinical isolate of *K pneumoniae* was obtained from the Clinical Microbiology Laboratory, Carolinas Medical Center, Charlotte, NC. Stock cultures of the bacterium were maintained in tryptic soy broth (TSB) medium plus 10% glycerol and stored at -80°C until use.

Animals

Female Swiss/Webster mice (Harlan Sprague-Dawley, Indianapolis, Ind) aged 10 to 11 weeks were housed in barrier conditions with controlled temperature and light cycle and having ad libitum access to food and water. All experiments were approved by the Atlanta University Center Institutional Animal Care and Use Committee and were carried out under the supervision of a veterinarian.

Experimental groups

Animals were randomly assigned to 3 groups ($n = 8/\text{group}$): (1) hindlimb-unloaded mice were suspended by the tail at 15° to 20° head-down tilt with no load bearing on hindlimb¹⁴; (2) restrained mice were restrained by the tail parallel to the ground, and all limbs were allowed to touch the floor of the cage; and (3) control mice were housed individually in standard caging. Animals in all groups were infected 2 days after the initiation of treatment. Additional uninfected control mice were maintained in individual cages. Experiments were repeated at least twice by using the same experimental conditions.

Inoculum preparation

For infection studies, samples of a previously frozen stock culture of *K pneumoniae* were inoculated into 10 mL of TSB medium for overnight growth at 37°C in 5% CO_2 . One hundred microliters of the stationary phase culture of the overnight growth was transferred to 10 mL of TSB medium, and standard growth curve fits were prepared by plotting absorbance readings at wavelength 595 versus the corresponding bacterial counts on agar plates; these were expressed as colony-forming units (CFUs).

For the 50% lethal dose (LD_{50}) determination, bacteria were grown to midlog phase in TSB medium for 4 hours at 37°C with gentle shaking. Cells were harvested and washed twice with PBS by spinning at 3000g for 10 minutes. Cell pellets were subsequently resuspended in 10 mL of PBS and serially diluted to the desired concentrations. Mice were intraperitoneally inoculated with doses ranging from 1×10^7 to 1×10^3 CFU/mL, and the LD_{50} was determined to be 4.6×10^6 CFU/mL by using a Reed-Muench estimation.²⁰ Concentrations were confirmed by plating 10-fold dilutions of the suspension on tryptic soy agar (TSA) solid medium.

Experimental infection and survival evaluation

Each mouse received 100 μL of bacterial suspension containing the previously determined LD_{50} dose administered through the intraperitoneal route 48 hours after suspension and restrain treatments to assess survival of mice in the 3 experimental groups. A sample of the *K pneumoniae* suspension was serially diluted and plated on TSA solid medium to determine the actual dose of bacteria injected into each mouse. Animal survival was assessed twice a day for 15 days.

Bacterial organ load studies

For bacterial counts, spleen, lungs, liver, and kidneys were aseptically removed from dead mice and placed in 5 mL of sterile PBS. Tissues were homogenized with a tissue homogenizer. One-hundred-microliter samples of three 1:10 serial dilutions were plated in TSA medium and incubated for 24 hours at 37°C . The number of CFUs was determined by averaging the number of colonies present on the 3 replicate serially diluted TSA plates.

Kinetics of bacterial growth in organs

Mice ($n = 8/\text{group}$) were inoculated intraperitoneally with a lower, nonlethal doses of *K pneumoniae* (1×10^6 CFU/mL) to determine the kinetics of bacterial growth and to decrease the rate of mortality observed after using the LD_{50} dose. At least 2 mice from each group were killed by means of cervical dislocation at different time points (6, 21, and 30 hours) after inoculation. Blood was collected through cardiac puncture, and organs were removed and processed, as described previously.

Statistical analysis

At least 2 separate experiments for each determination were performed in this study. Data were analyzed by using Statview 5.0.1, with the significance set at a P value of less than .05. Results were expressed as percentage of survival at each time point, as determined with the Kaplan-Meier method. Differences in survival rates between the groups were compared by using the Mantel-Cox log-rank test. The Student t test was used to test statistical significance between any 2 groups, and ANOVA was used for differences among more than 2 groups.

RESULTS

Hindlimb unloading results in decreased survival of mice infected with *K pneumoniae*

As shown in Fig 1, intraperitoneal inoculation of mice with one LD_{50} of bacteria resulted in a significant increase of mortality 2 to 4 days after inoculation in hindlimb-unloaded mice compared with restrained and normally housed control mice. A cumulative mortality rate of about 90% was observed by day 4 after infection in hindlimb-unloaded mice. Mortality rates in the range of 50% to 60% were observed in restrained and infected control mice, respectively, 3 days after inoculation. There were significant differences in the mortality rates between hindlimb-unloaded mice and both the restrained and control groups ($P < .032$). There was no significant difference in mortality rate between the restrained and control groups. In addition, the hindlimb-suspended group showed a significant decrease ($P < .0001$) in the mean day of death after infection (4.00 ± 0.23) compared with restrained mice (9.05 ± 0.45) and normally housed

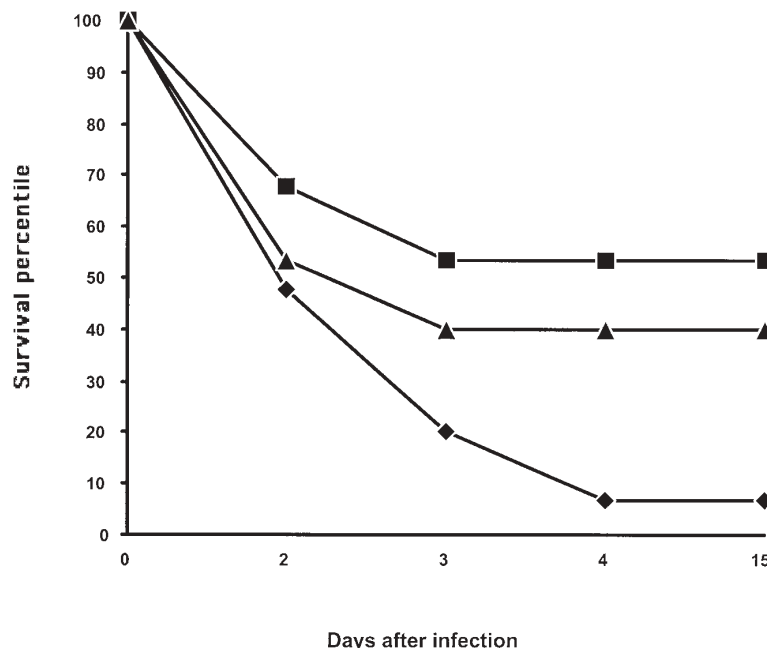


FIG 1. Effect of hindlimb unloading on survival of mice after intraperitoneal administration of *K pneumoniae* 2 days after treatment. Data were expressed as percentage survival at each time point, as determined by using the Kaplan-Meier method. Differences in survival among the 3 experimental groups of mice were compared by using the Mantel-Cox log-rank test. *Squares*, Hindlimb-unloaded and infected mice; *circles*, restrained and infected mice; *triangles*, normally caged control and infected mice.

TABLE 1. Bacterial load in organs isolated from mice infected with one LD₅₀ and subjected to different treatments*

Treatment	Spleen	Liver	Lungs	Kidney
Hindlimb-unloaded and infected mice	8.11 ± 0.16†	8.70 ± 0.12	8.61 ± 0.13	8.08 ± 0.17
Restrained and infected mice	7.94 ± 0.26	8.71 ± 0.17	8.68 ± 0.21	8.19 ± 0.21
Control and infected mice	7.88 ± 0.19	8.66 ± 0.19	8.42 ± 0.18	8.01 ± 0.16

*Hindlimb-unloaded, hindlimb-restrained, and control mice were intraperitoneally infected with *K pneumoniae* 2 days after the initiation of treatment. After death, organs were aseptically removed, homogenized in PBS, and plated in TSA solid medium at different dilutions. Bacterial load was determined as CFUs per milliliter.

†Each value represents the mean of log 10 of the CFUs per milliliter ± SE (n = 13 hindlimb-unloaded and infected mice, n = 7 restrained and infected mice, and n = 9 control and infected mice).

control mice (7.80 ± 0.70). No statistical differences were found in the mean time to death between restrained mice and normally caged control mice (Fig 2).

Bacterial organ load and bacterial dissemination in mice that did not survive

Quantitative culture of bacteria from lungs, spleen, liver, and kidneys removed from mice after death was carried out (Table I). No significant differences in the number of bacteria isolated from organs from mice that succumbed to infection among the 3 groups were detected. No bacteria were detected in organs of mice that survived the infection and were killed at the end of the experimental period.

Elevated bacterial organ load was detected in the hindlimb-unloaded group mice compared with control subjects 30 hours after inoculation

The dose of infection was reduced from 4.6×10^6 to 1×10^6 CFU/mL to decrease the rate of mortality to enable measurement of organ load in infected surviving mice. Bacterial dissemination in organs of mice from the 3 groups was evaluated as shown in Fig 3, A-F. Rapid bacterial spread was observed 6 hours after infection, with bacteria in the blood, liver, spleen, lungs, kidneys, and brain in all experimental groups. There were no significant differences among the groups at 6 and 21 hours after

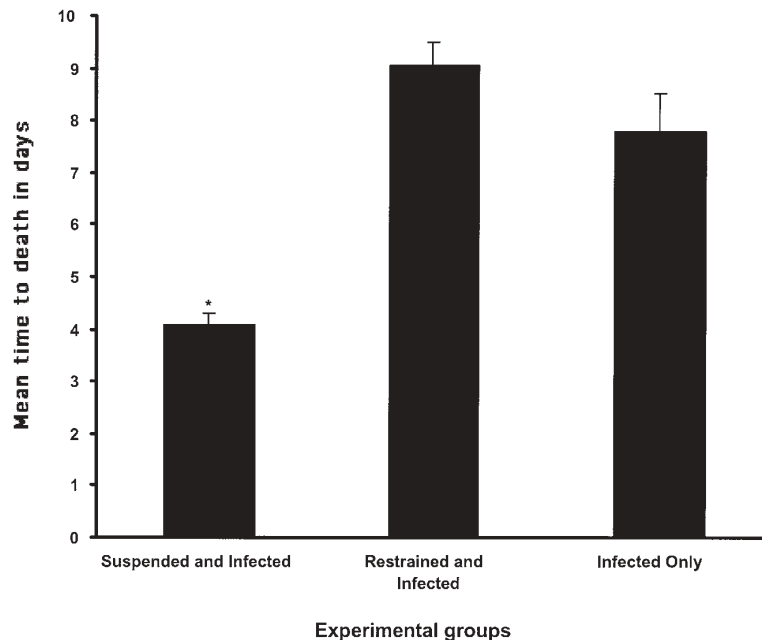


FIG 2. Average period to death of mice after intraperitoneal administration of *K pneumoniae* 2 days after hindlimb unloading. Each time point represents the mean $\pm$ SE, and values are expressed in days. *Statistically significant difference.

infection. However, by 30 hours after infection, a dramatic decrease in bacterial counts was observed in restrained and normally housed control mice, but the number of bacteria isolated from the organs and blood of hindlimb-unloaded mice continued to increase.

DISCUSSION

Hindlimb unloading of rodents has been shown to alter immunologic responses in a similar fashion to spaceflight.^{14,16,18} In fact, hindlimb suspension has been shown to alter resistance to both viral and bacterial infections in mice, but the organisms studied were the D variant encephalomyocarditis virus²¹ and *Listeria monocytogenes*.^{22,23} Neither of these organisms would be a likely human pathogen to be encountered during a spaceflight mission.

The present study was designed to assess the effects of hindlimb suspension on resistance of mice to *K pneumoniae*, a potent human pathogen that has been shown to cause infections in individuals exposed to severe stress, including sepsis in individuals exposed to severe trauma.¹⁹ Additional studies were carried out to determine potential mechanisms involved in hindlimb unloading–induced alterations in resistance to infection. The results indicate that infection of hindlimb-unloaded mice with *K pneumoniae* enhanced the development of sepsis and death caused by systemic infection. It is clear that all mice in all groups died from the same type of infectious process but that hindlimb-suspended mice had much greater susceptibility to the infectious process. Infected mice that succumbed could not clear the infection, and hindlimb-unloaded mice were much less likely to clear the infection than restrained or normally housed mice.

It is well documented that the innate host immune response is a very important host defense mechanism against bacterial pathogens, including *K pneumoniae*. Several chemokines and cytokines, such as IL-6 and macrophage inflammatory protein 2, are crucial for migration of leukocytes to infections sites and for bacterial clearance.²⁴ In addition to the immune system, the liver plays a major role in the clearance of bacterial pathogens from the host.²⁵ In the present study the effect of hindlimb unloading on infection and survival was targeted, but the mechanisms by which hindlimb unloading affects the immune system, resulting in increased mortality after infection, remain to be established. The present study suggests that hindlimb-unloaded mice might have compromised function of the immune system, especially in the recruitment and influx of granulocytes and activation of macrophages. Impairment of protective host immunity in suspended mice could be due to alteration in neutrophil and Kupffer cell interactions,²⁵ alteration of adherence molecules on the vascular endothelium,²⁶ activation of complement,²⁷ release of chemokines-cytokines,²⁴ and use of toll-like receptors.²⁸ Another possible mechanism that might contribute to the enhanced mortality and elevated bacterial growth is hepatic dysfunction from systemic endotoxemia and release of toxic mediators of inflammatory cytokines and oxidants of Kupffer cells resulting from hindlimb unloading.²⁹ Furthermore, hindlimb unloading might also lead to systemic endotoxemia, which could contribute to perturbation of cell membrane integrity and intestinal permeability and result in bacterial translocation from the gut. This could activate the Kupffer cells to release toxic mediators that would alter bacterial clearance and killing processes in the host.³⁰

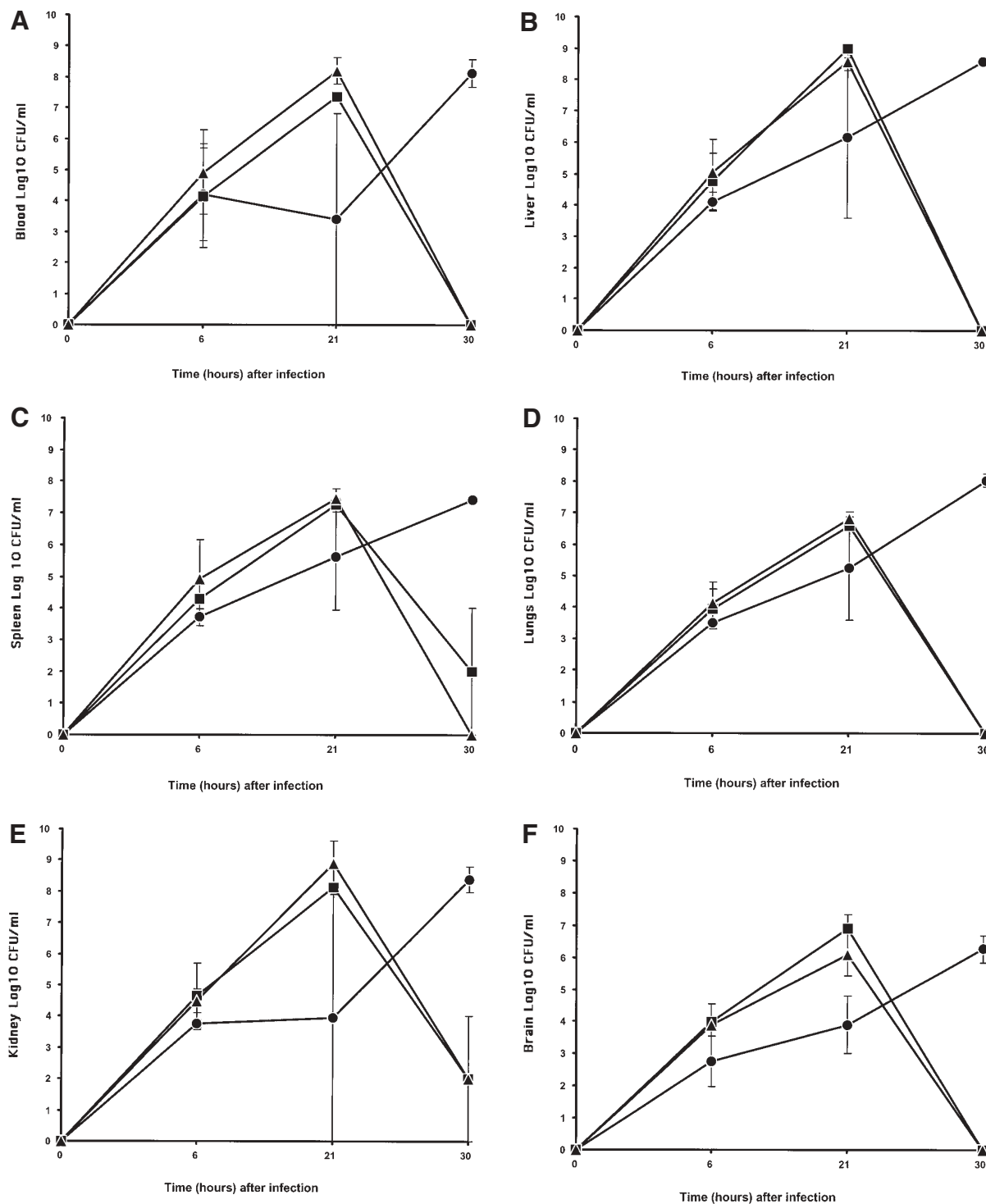


FIG 3. Organ distribution of *K pneumoniae* in mice 2 days after hindlimb unloading. CFU counts in bloods (A), liver (B), spleen (C), lung (D), kidney (E), and brains (F). Each time point represents the mean $\pm$ SE of CFUs per milliliter of 2 or more mice. Squares, Hindlimb-unloaded and infected mice; circles, restrained and infected mice; triangles, normally caged control and infected mice.

Stress could play a role in the altered resistance of hindlimb-unloaded mice to infection with *K pneumoniae*. Stress has been shown to alter immunologic param-

eters,¹⁻³ and this could certainly contribute to the decreased resistance to infection observed in the hindlimb-unloaded mice. It is possible that cytokines and

other neuroendocrine stress hormones are involved. It has been recently shown that IL-6 is crucial for an efficient defense against severe infections with gram-negative bacteria, including *K pneumoniae*.³¹ Hindlimb unloading has also been shown to alter cytokine production.²³ The influence of hindlimb unloading might have led to a lack of recruitment and activation of inflammatory cells by chemotactic factors. It is possible that the increased resistance to *K pneumoniae* infection and the capacity of the control mice and all survivors to clear bacteria and resolve the infection resulted from a normal activity of macrophages and neutrophils.

Additional direct effects of neuroendocrine hormones on bacterial growth and expression of virulence factors could play a role. Catecholamine levels have been shown to be increased in individuals who have undergone spaceflights.³² Catecholamines have also been shown to directly enhance the growth and expression of virulence factors of a variety of gram-negative bacteria, including *K pneumoniae*.³³⁻⁴¹ Additionally, in vitro growth in spaceflight conditions has been shown to affect virulence of gram-negative bacteria.⁴² It is possible that a combination of suppression of immune responses and enhancement of bacterial growth and virulence could play a role in the compromised resistance of hindlimb-unloaded mice to infection.

It is likely, however, that stress alone cannot account for the compromised resistance to infection of the hindlimb-unloaded mice. Mice that were restrained in the current study did not show compromised resistance to infection. The hindlimb-unloading model, with its simulation of some spaceflight conditions, including muscle and bone loss and fluid shift to the head, was required to compromise resistance to infection. Therefore it is possible that spaceflight conditions combine with stress to result in compromised resistance to infection. This is certainly an area worthy of further study to insure the safety of potential space travelers, particularly as more flight opportunities develop with much longer exposure times to spaceflight conditions in the International Space Station era.

REFERENCES

- Peterson K, Phillip CC, Chao F, Molitor T, Murtaugh M, Strgar F, et al. Stress and pathogenesis of infectious diseases. *Rev Infect Dis* 1991;13:710-20.
- Solomon GF. Psychoneurology: interactions between central nervous system and immune system. *J Neurosci Res* 1987;18:1-9.
- Weizman R, Bessler H. Cytokines: stress and immunity—an overview. In: Plotnikoff NP, Faith RE, Murgo AJ, Good RA, editors. *Cytokines stress and immunity*. Boca Raton (FL): CRC Press LLC; 1999. p. 1-17.
- Aviles H, Monroy FP. Immunomodulatory effects of cold stress on mice infected intraperitoneally with a 50% lethal dose of *Toxoplasma gondii*. *Neuroimmunomodulation* 2001;9:6-12.
- Aviles H, Monroy FP. *Toxoplasma gondii*: cold stress-induced modulation of antibody. *Exp Parasitol* 2001;9:6-12.
- Konstantinova IV, Rykova MP, Lesnyak AT, Antropova EA. Immune changes during long-duration missions. *J Leukoc Biol* 1993;54:189-201.
- Sonnenfeld G, Mandel ED, Konstantinova IV, Berry WD, Taylor GR, Lesnyak AT, et al. Spaceflight alters immune cell function and distribution. *J Appl Physiol* 1993;17:191S-5S.
- Sonnenfeld G, Mandel AD, Konstantinova IV, Taylor GR, Berry WD, Welhausen SR, et al. Effects of spaceflight on levels and activity of immune cells. *Aviat Space Environ Med* 1990;61:648-53.
- Taylor GR, Dargado JR. Human cellular responsiveness following spaceflight. *Aviat Space Environ Med* 1983;54(suppl 1):S55-9.
- Taylor GR, Neale LS, Dardano JR. Immunological analysis of U.S. space shuttle crew members. *Aviat Space Environ Med* 1984;57:213-7.
- Durnova GN, Kaplansky AS, Portuglov VV. Effects of a 22-day spaceflight on the lymphoid organs of rats. *Aviat Space Environ Med* 1976;47:588-91.
- Mandel AD, Balish E. Effect of spaceflight on cell mediated immunity. *Aviat Space Environ Med* 1976;48:1051-7.
- Morey E. Space flight and bone marrow correlation with a new model of weightlessness. *Bioscience* 1999;29:168-74.
- Chapes SK, Mastro AM, Sonnenfeld G, Berry WD. Antiorthostatic suspension as a model for the effects of space flight on immune system. *J Leukoc Biol* 1993;54:227-35.
- Steffen JM, Robb R, Dambrowski MJ, Musacchia XJ, Mandel AD, Sonnenfeld G. A suspension for hypokinetin/hypodynamic and antiothostatic responses in the mouse. *Aviat Space Environ Med* 1984;55:612-6.
- Steffen JM, Musacchia XJ. Thymic involution in the suspended rat. Model for weightlessness: decreased glucocorticoid receptor concentration. *Physiologist* 1984;27(suppl):S39-40.
- Zerath E. Effects of microgravity on bone and calcium homeostasis. *Adv Space Res* 1998;21:1049-58.
- Sonnenfeld G, Morey ER, Williams JA, Mandel AJ. Effect of a stimulated microgravity model on the production of rat interferon. *J Interferon Res* 1982;2:467-70.
- Polk HC Jr, Cheadle WG, Sonnenfeld G, Hershman MJ. Infection associated with the surgical care of the major trauma victim. In: Jaffe H, Bucalo B, Sherwin S, editors. *Antiinfective application of interferon-gamma*. New York: Marcel Dekker; 1992. p. 29-36.
- Reed LJ, Muench H. A simple method of estimating fifty percent end points. *Am J Hyg* 1938;27:493-7.
- Gould CL, Sonnenfeld G. Enhancement of viral pathogenesis in mice maintained in an antiothostatic suspension model: Coordination with effects on interferon production. *J Biol Regul Homeost Agents* 1987;1:33-6.
- Miller ES, Sonnenfeld G. Influence of antiothostatic suspension on resistance to murine *Listeria monocytogenes* infection. *J Leukoc Biol* 1994;55:371-8.
- Miller ES, Bates RA, Koebel DA, Sonnenfeld G. Antiothostatic suspension stimulates profiles of macrophage activation in mice. *Neuroimmunomodulation* 1999;6:160-7.
- Greenberger MJ, Streiter RM, Kunkel SL, Danforth JM, Laichalk LL, McGillicuddy DC, et al. Neutralization of macrophage inflammatory protein-2 attenuated neutrophil recruitment and bacterial clearance in murine *Klebsiella pneumoniae*. *J Infect Dis* 1996;173:159-65.
- Gregory SH, Cousens LP, Rooijen NV, Dopp EdA, Carlos TM, Wing EJ. Complementary adhesion molecules promote neutrophil-Kupffer cell interaction and the elimination of bacteria taken up by the liver. *J Immunol* 2002;168:308-15.
- O'Brien AD, Standiford TJ, Bucknell KA, Wilcoxon SE, Paine R 3rd. Role of alveolar epithelial intracellular adhesion molecule-1 in host defense against *Klebsiella pneumoniae*. *Am J Physiol* 1999;276:L961-70.
- Taylor PW. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. *Micriobiol Rev* 1983;47:46-83.
- Roger T, David J, Glauser MP, Calandra T. MIF regulates innate immune responses through modulation of toll-like receptor 4. *Nature* 2001;414:920-4.
- Brunner LJ, Dipiro JT, Feldman S. Antipyrene pharmacokinetics in the tail-suspended rat model. *J Pharmacol Exp Ther* 1995;274:345-52.
- Amidon GL, DeBrincat GA, Najib N. Effects of gravity on gastric emptying, intestinal transit and drug absorption. *J Clin Pharmacol* 1991;31:968-73.
- Enckevort FH, Sweep CG, Span PN, Netea MG, Hermus AR, Kullberg BJ. Reduced adrenal response and increased mortality after systemic *Klebsiella pneumoniae* infection in interleukin-6-deficient mice. *Eur Cytokine Netw* 2001;12:581-6.
- Christensen NJ, Bratholm P, Knudsen JH. Lymphocyte norepinephrine and ephinephrine as indices of long-term changes in sympathoadrenal activity in human subjects. *J Gravitational Physiol* 1997;4:115-6.
- Sheridan JF, Dobbs C, Jung J, Chu X, Konstantinos A, Padgett D, et al. Stress-induced neuroendocrine modulation of viral pathogenesis and immunity. *Ann N Y Acad Sci* 1998;840:803-8.
- Kopydlowski KM, Mcvey DS, Woos KM, Landolo JJ, Chapes SK.

- Effects of antiorthostatic suspension and corticosterone on macrophage and spleen cell function. *J Leuk Biol* 1992;52:202-8.
35. Lyte M. The role of microbial endocrinology in infectious disease. *J Endocrinol* 1993;137:343-5.
 36. Kinney SK, Austin CE, Morton DS, Sonnenfeld G. Catecholamine enhancement of *Aeromonas hydrophila* growth. *Microbial Pathogenesis* 1999;26:85-91.
 37. Lyte M, Ernst S. Catecholamine induced growth of gram negative bacteria. *Life Sci* 1992;50:203-13.
 38. Lyte M, Bailey MT. Neuroendocrine-bacterial interactions in a neutrotoxin-induced model of trauma. *J Surg Res* 1997;70:195-201.
 39. Neal CP, Freestone PP, Maggs E, Haigh AF, Williams RD, Lyte M. Catecholamine inotropes as growth factors for staphylococcus epidermidis and other coagulase-negative staphylococci. *FEMS Microbiol Lett* 2001;194:163-9.
 40. Greenberger MJ, Streiter RM, Kunkel SL, Danforth JM, Laichalk LL, McGillicuddy DC, et al. Neutralization of macrophage inflammatory protein-2 attenuates neutrophil recruitment and bacterial clearance in murine *Klebsiella pneumoniae*. *J Infect Dis* 1996;173:159-65.
 41. Belay T, Sonnenfeld G. Differential effects of catecholamines on in vitro growth of pathogenic bacteria. *Life Sci* 2002;71:447-56.
 42. Nickerson CA, Ott CM, Mister SJ, Morroe BJ, Burns-Keliher L, Pierson DL. Microgravity as a novel environmental signal affecting *Salmonella enterica* serovar Typhimurium virulence. *Infect Immun* 2000;68:3147-52.

Bound volumes available to subscribers

Bound volumes of The Journal of Allergy and Clinical Immunology are available to subscribers (only) for the 2002 issues from the Publisher, at a cost of \$107.00 for domestic, and \$128.00 for international subscribers for Vol. 109 (January-June) and Vol. 110 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby, Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887; phone (800) 654-2452 or (407) 345-4000.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.