Published in final edited form as:

Radiat Res. 2011 April ; 175(4): 485–492. doi:10.1667/RR2373.1.

Effect of Solar Particle Event Radiation on Gastrointestinal Tract Bacterial Translocation and Immune Activation

Houping Ni^a, Klara Balint^a, Yu Zhou^a, Daila S. Gridley^c, Casey Maks^b, Ann R. Kennedy^b, and Drew Weissman^{a,1}

^aDivision of Infectious Diseases, Department of Medicine

^bDepartment of Radiation Oncology, University of Pennsylvania, 3610 Hamilton Walk, Philadelphia, Pennsylvania 19104

^cDepartment of Radiation Medicine, Loma Linda University & Medical Center, Loma Linda, California 92354

Abstract

Space flight conditions within the protection of Earth's gravitational field have been shown to alter immune responses, which could lead to potentially detrimental pathology. An additional risk of extended space travel outside the Earth's gravitational field is the effect of solar particle event (SPE) radiation exposure on the immune system. Organisms that could lead to infection include endogenous, latent viruses, colonizing pathogens, and commensals, as well as exogenous microbes present in the spacecraft or other astronauts. In this report, the effect of SPE-like radiation on containment of commensal bacteria and the innate immune response induced by its breakdown was investigated at the radiation energies, doses and dose rates expected during an extravehicular excursion outside the Earth's gravitational field. A transient increase in serum lipopolysaccharide was observed 1 day after irradiation and was accompanied by an increase in acute-phase reactants and circulating proinflammatory cytokines, indicating immune activation. Baseline levels were reestablished by 5 days postirradiation. These findings suggest that astronauts exposed to SPE radiation could have impaired containment of colonizing bacteria and associated immune activation.

INTRODUCTION

The risks for developing infections during space flight include reduced weight bearing [reviewed in ref. (1)], stress (2), radiation (3, 4), disturbance of circadian rhythms (5), and altered nutritional intake (6). These risks, as well as disturbances caused by the interactions between the physiological systems that are altered during space flight, pose a threat for development of pathogenic infection by endogenous or exogenous organisms. Endogenous organisms, which are resident in the astronaut at the start of space flight, consist of latent viruses common in humans (e.g., Epstein-Barr, Herpes simplex and others) or commensal and colonizing pathogenic organisms, while exogenous organisms are present in the spacecraft and other astronauts (3, 7–9). A large body of literature exists on the impairment of the immune system in space flight models with a reduced ability to control infections, suppression of bone marrow function, and altered acquired immunity [reviewed in ref. (10)]. Fifteen of 29 Apollo astronauts contracted bacterial or viral infections either during their

missions or within a week of returning, although none were severe. A urinary tract infection with *Pseudomonas aeruginosa* was documented after an Apollo mission (11). Increased ear and skin infections were noted during early space missions (12). A reduced ability to clear infections by *Klebsiella pneumonia* (13) and *Pseudomonas aeruginosa* (14) has been reported in studies using animal models of space flight. Resistant mice have been shown to develop infections with the Encephalomyocarditis virus D variant when they were subjected to hind limb unloading (15). The decrease in resistance to infection was correlated with a drop in type I interferon production (16). In another model of space flight using murine infection with *Listeria monocytogenes*, innate immunity based resistance to primary infection was enhanced, but the ability to generate a T-lymphocyte immune response was severely impaired (17). Decreased antibiotic potency and enhanced microbial virulence associated with space flight (18) could further increase the risk for serious infections in immunocompromised astronauts. With the prospect of extended missions outside of the Earth's gravitational field, the effect of galactic emissions on immune function and the risk of infection needs to be evaluated further.

The primary components of interplanetary radiation are galactic cosmic rays and solar radiation (19), which consists of low-energy solar wind and highly energetic solar particle events (SPEs) that originate from magnetically disturbed regions of the sun (20, 21). SPEs are unpredictable and typically last for no more than several hours, although some may continue for several days. SPEs are composed predominately of low-energy protons with a minor contribution from helium ions (~10%) and an even smaller contribution from heavy ions and electrons (~1%).

Astronauts are at greatest risk for radiation exposure from SPEs while performing extravehicular activities. It is predicted that astronauts may receive up to a dose of 2 Gy to the bone marrow (22) and up to 25 Gy to the skin (23). While it is expected that a 2-Gy bone marrow dose of conventional radiation (electrons, X or γ rays) is likely to have adverse effects on immune function, it is not known whether 2 Gy of protons given at dose rates expected during an SPE (1–50 cGy/h) will have similar effects. Thus this study examined the effect of SPE-like radiation on the containment of commensal bacteria and the immune response developed as a consequence of its breakdown.

MATERIALS AND METHODS

Irradiation of Mice

The Institutional Animal Care and Use Committees at the University of Pennsylvania and Loma Linda University Medical Center (LLUMC) approved all animal studies. Male and female outbred ICR mice, 5–6 weeks of age, were obtained from Harlan Laboratories (Livermore, CA). For irradiation, the mice were placed in aerated plastic chambers (AMAC #530C) with dimensions of 7.30 cm $\times$ 4.13 cm $\times$ 4.13 cm. The chambers allowed the mice to turn around easily (reverse nose to tail direction). The mice were exposed to total-body radiation with 50 or 70 MeV protons delivered in a spread-out Bragg peak (SOBP) at doses of 50 cGy, 1 Gy and 2 Gy using the horizontal clinical beam line at LLUMC. Gamma radiation was delivered using a ^{60}Co source (Eldorado Model 'G' machine, Atomic Energy of Canada Ltd, Commercial Products Division, Ottawa, Canada). The γ -ray and proton exposures were delivered in a single fraction at low (50 cGy/h) or high (50 cGy/min) dose rates. The radiation dosimetry details will be described elsewhere (Maks *et al.*, manuscript in preparation).

Groups of four to five mice per treatment were used. Blood was obtained 1, 5, 11 and 23 days after irradiation by cheek lancet. Serum was separated by centrifugation at 4,000 rpm

for 4 min in an Eppendorf microfuge. Ten-millimeter skin biopsies of the posterior flank were obtained at 2, 6, 12 and 24 days after irradiation and frozen at -80°C .

Lipopolysaccharide (LPS) Assay

Serum LPS was measured with two different assays. The first was a standard end point chromogenic LAL assay (Lonza, Walkersville, Inc., Walkersville, MO) with a sensitivity range of 0.1 to 1.0 EU/ml (20 to 200 pg/ml). Serum was diluted 1 to 5 with endotoxin-free water and heat-inactivated at 80°C for 15 min. The inhibitory activity at this dilution was reduced but was still apparent and was variable across samples. A second assay that uses the first component of the LAL reaction, Factor C (Lonza), with a sensitivity range of 0.01 to 10 EU/ml (2 to 2000 pg/ml) was also used. Serum was diluted 1 to 20 in endotoxin-free water, which reduced inhibitory activity to negligible levels after heating to 80°C for 15 min. At this dilution, the lower limit of detection of the assay was 40 pg/ml, and all samples with values less than this were assigned this value. Samples were run in duplicate.

LPS Binding Protein (LBP) and Soluble (s)CD14 ELISAs

Serum was analyzed for LBP and sCD14 by direct binding ELISA (Cell Sciences, Canton, MA), as described by the manufacturer. Serum was diluted 1:500 with PBS and analyzed in duplicate for LBP. Serum was diluted 1:100 with PBS and analyzed in duplicate for sCD14.

Cytokine Elution from Skin

Ten-millimeter full-thickness skin biopsies were weighed and 1 ml of ice-cold PBS containing 0.1% Igepal CA-630 (Sigma, St. Louis, MO) nonionic detergent was added for 10 min followed by homogenizing the tissues with a tissue disrupter (Wheaton Science Products, Millville, NJ) (24). The tissue was centrifuged for 5 min at 8,000 rpm in a microfuge and the supernatants were stored at -80°C .

Luminex Cytokine Analyses

Proinflammatory cytokines in serum and skin eluate were measured with the Inflammatory Cytokine Mouse 4-Plex Panel (Invitrogen, Carlsbad, CA) using a Luminex 100 reader (Luminex Corporation, Austin, TX). Skin eluate and serum were analyzed without dilution. The lower limit of detection of the assay was 7.3 pg/ml for interleukin- 1β (IL- 1β), 6.5 pg/ml for IL-6, and 7.8 pg/ml for tumor necrosis factor- α (TNF- α); all samples with values less than this were assigned these values. Samples were assayed in duplicate.

Immunohistochemistry

Terminal ileum from animals 2 or 7 days postirradiation was snap frozen in OCT medium (Thermo Fisher, Rockford, IL) and kept at -80°C . Tissue was cut into 6- μm sections in a Leica CM1850 cryostat (Bannockburn, IL), fixed in acetone (Thermo Fisher), stained with rabbit anti-human Claudin-3 antibodies or control rabbit Ig (Thermo Fisher), and detected with biotinylated goat anti-rabbit IgG (Biocare Medical, Concord, CA), streptavidin horseradish-peroxidase (Vector, Burlingame, CA), and AEC substrate (Sigma). Tissue was counter-stained with hematoxylin (Electron Microscopy Sciences, Hatfield, PA) and examined and photographed under bright-field microscopy (Nikon Eclipse e1000, Melville, NY). Quantification of breaks in Claudin-3 staining were made by counting the number of breaks per 1000 enterocytes in tissue from five animals in each treatment group. Goblet cell counts were used as a control.

Statistics

Means and SEM were determined and paired Student's *t* tests were performed using Microsoft Excel software.

RESULTS

The GI tract contains over 10^{12} bacteria whose functions include carbohydrate fermentation and absorption, repression of pathogenic microbial growth, metabolic activity, and continuous and dynamic effects on the gut and systemic immune system. The control of bacteria and bacterial product passage across the GI mucosa, known as translocation, is an important function that can be disturbed in multiple diseases (25, 26). The effect of SPE-like radiation on GI containment of bacterial products was analyzed using proton radiation at energies (50 and 70 MeV SOBPs), doses (0.5, 1 and 2 Gy) and dose rates (50 cGy/h) possible during a typical strong SPE. For comparison, the same total doses of γ and proton radiation at were delivered at a high dose rate (50 cGy/min). It is expected that the maximum dose delivered to the bone marrow during an SPE would not exceed 2 Gy; thus the maximum dose delivered in this study was 2 Gy.

SPE-Like Radiation Exposure Causes Bacterial Product Translocation Across the GI Tract

Increased levels of circulating LPS were found 24 h after a 2-Gy low-dose-rate (50 cGy/h) proton exposure (Fig. 1A). A similar increase was observed for high-dose-rate (50 cGy/min) protons (Fig. 1A) and for 2 Gy of γ radiation at low and high dose rates (Fig. 1B). No increase in circulating LPS was detected 5 days after irradiation (Fig. 1C and data not shown). To exclude the possibility that oral bacteria-derived LPS was contaminating our samples, blood from the tail vein was compared to cheek pouch-derived blood with similar results. Two different assays were used to measure serum LPS, an end point kinetic Limulus amebocyte lysate (LAL) assay and a more sensitive assay of recombinant Factor C, which is the first component of the LAL response. Serum diluted 1:5 had produced significant and variable inhibition of LPS after heat inactivation, while a 1:20 dilution had minimal inhibition. Because the cutoff value for the Factor C assay at this dilution was 40 pg/ml of LPS (0.2 EU/ml), any sample with less than 40 pg/ml of LPS was set to 40 pg/ml. Similar results were found with the end point LAL assay, but because a 1:5 dilution was needed to measure serum LPS levels, the results were variable. We studied both male and female mice and used both 50 MeV and 70 MeV protons and found no differences in the increases in circulating LPS or in the other assays and hence combined the data for the analyses. Control animals placed in boxes for the same amount of time as the irradiated animals but not irradiated were similar to control mice. The low-dose-rate groups started their radiation exposure 4 h before the high-dose-rate groups, but, since we did not see any difference between low and high dose rates at 24 h after completion of irradiation and since sham-irradiated controls for the total time for both low and high dose rate were the same, we concluded that the additional time required for the low-dose-rate irradiation did not alter the measurements of effect. There were no increases in the levels of circulating LPS at radiation doses below 2 Gy compared to the boxed control groups.

SPE-Like Radiation Induces a Transient Acute-Phase Response

An increase in circulating LPS can induce the release of acute-phase reactants that serve multiple functions, including inhibiting pathogen growth, promoting opsonization, and recruiting immune cells (27). LPS binding protein (LBP) is an early acute-phase reactant that is required for the innate immune responses to LPS (28). Circulating LBP was increased 1 day after low-dose-rate proton irradiation (Fig. 2A). As observed for LPS, the increase in LBP also occurred with high-dose-rate proton radiation and with both low- and high-dose-rate γ radiation; a statistically significant increase was noted only with the highest dose of 2 Gy (Fig. 2A, B). By 5 days postirradiation, the levels returned to and remained at the level observed in the corresponding unirradiated boxed controls. No differences in serum LBP in controls restrained in boxes for 4, 2 or 1 h, corresponding to 2 Gy, 1 Gy and 50 cGy, respectively, and unrestrained mice were observed.

SPE-Like Radiation Induces Elevation of Circulating Soluble CD14

CD14 is the LPS binding receptor that presents LPS to the signaling receptor complex comprised of myeloid differentiation protein 2 (MD-2) and Toll-like receptor 4 (TLR4). It is found in both glycosylphosphatidylinositol (GPI)-linked cell surface and soluble forms (29). Increased levels of sCD14 are found during infection and sepsis, and the delivery of sCD14 with LPS reduces the lethal effects of LPS (30). It is also the most sensitive measure of increased bacterial translocation. One day after exposure to 2 Gy proton or γ radiation given at low or high dose rates, a significant increase in the serum levels of sCD14 occurred that returned to control levels by 5 days postirradiation (Fig. 3 and data not shown). The control group shown was restrained in boxes for 4 h, which corresponded to the time the 2-Gy low-dose-rate groups were restrained.

SPE-Like Radiation Induces a Transient Increase in Circulating Proinflammatory Cytokines

The observation that 2 Gy of proton or γ radiation led to a transient increase in bacterial translocation leading to activation of an acute-phase response led us to investigate whether a systemic effect on the innate immune system occurred. The proinflammatory cytokines TNF- α , IL-1 β and IL-6 were measured in serum, and a small transient increase 1 day after proton or γ irradiation at low and high dose rates was found that returned to baseline levels 5 days after irradiation (Fig. 4 and data not shown). An astronaut exposed to an SPE could receive over 25 Gy of radiation to the skin (23). We assessed whether proton or reference γ radiation delivered at low or high dose rates led to measurable levels of proinflammatory cytokines in the skin. No significant increase was observed in the skin 2 days after irradiation (data not shown).

SPE-Like Radiation Induces Transient Disruption of Tight Junctions in the GI Tract

Disease states associated with bacterial translocation are associated with a breakdown in the integrity of the epithelial layer of the GI tract (31). Terminal ileum was obtained from control mice or mice irradiated with 2 Gy high- or low-dose-rate protons or γ rays 2 or 7 days postirradiation. Immunohistochemistry for the tight junction protein Claudin-3 demonstrated occasional regions of reduced staining and interruptions along the epithelial barrier at 2 days postirradiation (Fig. 5). Such disruptions were observed at a frequency of 0.71 to 1.12 occurrences per 1,000 enterocytes and were similar at high or low dose rate in proton- or γ -irradiated tissue (Table 1). Claudin-3 staining in control and irradiated tissue was not significantly different on day 7.

DISCUSSION

Future space missions will involve extended time outside the Earth's gravitational field with the loss of its protection from galactic cosmic rays and solar radiation. In addition to the factors that influence immune function that are associated with space exploration circling the Earth, including reduced weight bearing (1), stress (2), disturbance of circadian rhythms (5), and altered nutritional intake (6), the effect of radiation from SPEs must also be considered. Astronauts could receive up to 2 Gy to the bone marrow from an SPE (22). This dose of proton radiation delivered at an energy and dose rate possible during an SPE results in a transient increase in the release of LPS into the circulation and potentially pathological changes to the GI tract. In our experiments, the increases in LPS were small, less than twofold, but were statistically significant. An increase in the acute-phase reactant LPS binding protein, which is required for LPS signaling, was also observed, as was a transient elevation in sCD14. Immunohistological analysis of GI tract tissue demonstrated occasional disruptions in the epithelial cell barrier as measured by a loss in tight junctions. Finally, the serum proinflammatory cytokines TNF- α , IL-1 β and IL-6 increased 1 day after irradiation, demonstrating systemic activation of the innate immune system.

The expression of proinflammatory cytokines induced by translocation of bacterial products across the GI tract can have multiple effects on immune function. The induction of inflammatory cytokines occurs in a finely tuned time-dependent equilibrium. They can have multiple and opposite effects in the same subject depending on the time of expression. The aberrant expression of proinflammatory cytokines can result in unusual and unwanted regulation of an immune response [reviewed in ref. (32)]. In an astronaut exposed to an SPE with resulting release of proinflammatory cytokines, exposure to a pathogenic insult could result in an altered immune response, resulting in incomplete protection from infection or immunopathology from an exaggerated response.

Previous studies of the effect of radiation on the immune system in space-flight models documented changes in cell numbers in immune organs and alterations of function based on *in vitro* analyses (3, 33–36), which included alterations in T, B and NK cell numbers in blood and spleen (33) and suppression of T-cell responses to mitogens (35). These studies give important information on radiation-induced immune function alterations but do not investigate or identify important *in vivo* interactions and mechanisms that could be responsible for the immune alterations measured *in vitro* or that could predict potential pathological sequelae. Elevations in circulating LPS with associated immune activation are found in HIV infection, Crohn's disease, systemic inflammatory response syndrome, and acute pancreatitis, all of which are associated with immune suppression, and the level of bacterial translocation is correlated with the amount of immune suppression (37). The observation of defects in tight junctions and epithelial cell integrity in the GI tract may be the mechanism of increased translocation, as has been suggested in HIV infection (31).

GI tract flora also affects the immune system in the gut. The first introduction of gut microbiota to an animal raised under axenic conditions results in a transient proinflammatory response that is replaced by an anti-inflammatory response by 7 days and is associated with inflammatory mononuclear cell infiltrates into the lamina propria (38). Effects of gut microbiota and the amount of LPS in the diet also affect the acquired immune response in the gut and draining lymphoid organs (39). We do not believe that the gut microbiota of SPF mice compared to feral mice would alter the responses to radiation we observed. Commensal bacteria have also been demonstrated to protect the GI tract from radiation-induced damage [reviewed in ref. (40)].

The consequences of radiation-induced damage to the GI tract are also relevant in clinical settings. One example is the GI tract injury that occurs in some cancer patients undergoing radiotherapy. Interactions between enteric microbes and the innate immune system have the potential to modulate the intestinal response to radiation, e.g., apoptosis and crypt survival (40). In murine models of bone marrow transplantation, increased translocation of LPS into the circulation and the dysregulation of inflammatory cytokine production have been shown to reciprocally increase GI damage (41). A study in which 5 Gy of total-body radiation of unspecified type and energy was delivered found a 10-fold increase in serum LPS with resulting activation of the innate immune system that led to an improvement in the function of adoptively transferred CD8⁺ T cells (42). These and other similar findings have led to the search for countermeasures, including innate immune system modulators, with potential to minimize deleterious effects (40).

The data presented in this study demonstrate that proton and reference γ radiation, whether delivered at dose rates expected during an SPE or 60 times higher, led to transient increases in bacterial translocation and immune activation. An impairment in the ability of the GI tract to exclude bacterial products and the resultant systemic activation of the immune system could have significant implications for astronaut safety during an extended mission in which an SPE occurred. Although the amount of bacterial translocation and immune activation

observed likely would not lead directly to infectious complications, and we did not find replication-competent bacteria in the blood of irradiated animals; the addition of other immune alterations caused by microgravity, stress, circadian rhythm alterations, and nutrition or effects on other organ systems, including the immune system, caused by SPE radiation could lead to more pronounced effects. Immune activation also impairs immune function, adding an additional decrement in function. Further studies to determine the effect and possible synergy of additional space-flight factors, particularly simulated hypogravity, on immune function are needed.

Acknowledgments

This work was supported by a grant from the National Space Biomedical Research Institute (NSBRI) through NASA NCC 9-58. We acknowledge the help of several CARR investigators in the irradiation of animals as part of these studies, including Jeffrey Ware, Ph.D. and Jenine Sanzari, Ph.D., from the University of Pennsylvania. In addition, we acknowledge the help of numerous investigators from the Loma Linda University Medical Center, including Andrew Wroe, Ph.D., Michael J. Pecaut, Ph.D., Adeola Y. Makinde, Ph.D., Steve Rightnar, Pete Koss, Tanya Freeman, Gordon Harding, Celso Perez and Melba Andres.

REFERENCES

1. Aponte VM, Finch DS, Klaus DM. Considerations for non-invasive in-flight monitoring of astronaut immune status with potential use of MEMS and NEMS devices. *Life Sci.* 2006; 79:1317–1333. [PubMed: 16757003]
2. Wang KX, Shi Y, Denhardt DT. Osteopontin regulates hindlimb-unloading-induced lymphoid organ atrophy and weight loss by modulating corticosteroid production. *Proc. Natl. Acad. Sci. USA.* 2007; 104:14777–14782. [PubMed: 17785423]
3. Shearer WT, Zhang S, Reuben JM, Lee BN, Butel JS. Effects of radiation and latent virus on immune responses in a space flight model. *J. Allergy Clin. Immunol.* 2005; 115:1297–1303. [PubMed: 15940150]
4. Uri JJ, Haven CP. Accomplishments in bioastronautics research aboard International Space Station. *Acta Astronaut.* 2005; 56:883–889. [PubMed: 15835037]
5. Mallis MM, DeRoshia CW. Circadian rhythms, sleep, and performance in space. *Aviat. Space Environ. Med.* 2005; 76:B94–B107. [PubMed: 15943202]
6. Cena H, Sculati M, Roggi C. Nutritional concerns and possible countermeasures to nutritional issues related to space flight. *Eur. J. Nutr.* 2003; 42:99–110. [PubMed: 12638031]
7. Taylor GR. Recovery of medically important microorganisms from Apollo astronauts. *Aerosp. Med.* 1974; 45:824–828. [PubMed: 4153037]
8. Novikova N, De Boever P, Poddubko S, Deshevaya E, Polikarpov N, Rakova N, Coninx I, Mergeay M. Survey of environmental biocontamination on board the International Space Station. *Res. Microbiol.* 2006; 157:5–12. [PubMed: 16364606]
9. Pierson DL, Stowe RP, Phillips TM, Lugg DJ, Mehta SK. Epstein-Barr virus shedding by astronauts during space flight. *Brain Behav. Immun.* 2005; 19:235–242. [PubMed: 15797312]
10. Borchers AT, Keen CL, Gershwin ME. Microgravity and immune responsiveness: implications for space travel. *Nutrition.* 2002; 18:889–898. [PubMed: 12361784]
11. Aviles H, Belay T, Fountain K, Vance M, Sonnenfeld G. Active hexose correlated compound enhances resistance to *Klebsiella pneumoniae* infection in mice in the hindlimb-unloading model of spaceflight conditions. *J. Appl. Physiol.* 2003; 95:491–496. [PubMed: 12692142]
12. Schmitt DA, Schaffar L. Isolation and confinement as a model for spaceflight immune changes. *J. Leukoc. Biol.* 1993; 54:209–213. [PubMed: 8371050]
13. 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; 110:262–268. [PubMed: 12170267]

14. Aviles H, Belay T, Fountain K, Vance M, Sonnenfeld G. Increased susceptibility to *Pseudomonas aeruginosa* infection under hindlimb-unloading conditions. *J. Appl. Physiol.* 2003; 95:73–80. [PubMed: 12626488]
15. Gould CL, Sonnenfeld G. Enhancement of viral pathogenesis in mice maintained in an antiorthostatic suspension model: coordination with effects on interferon production. *J. Biol. Regul. Homeost. Agents.* 1987; 1:33–36. [PubMed: 2461049]
16. Rose A, Steffen JM, Musacchia XJ, Mandel AD, Sonnenfeld G. Effect of antiorthostatic suspension on interferon-alpha/beta production by the mouse. *Proc. Soc. Exp. Biol. Med.* 1984; 177:253–256. [PubMed: 6483860]
17. Miller ES, Sonnenfeld G. Influence of antiorthostatic suspension on resistance to murine *Listeria monocytogenes* infection. *J. Leukoc. Biol.* 1994; 55:371–378. [PubMed: 8120454]
18. Klaus DM, Howard HN. Antibiotic efficacy and microbial virulence during space flight. *Trends Biotechnol.* 2006; 24:131–136. [PubMed: 16460819]
19. Hellweg CE, Baumstark-Khan C. Getting ready for the manned mission to Mars: the astronauts' risk from space radiation. *Naturwissenschaften.* 2007; 94:517–526. [PubMed: 17235598]
20. Wilson JW, Cucinotta FA, Shinn JL, Simonsen LC, Dubey RR, Jordan WR, Jones TD, Chang CK, Kim MY. Shielding from solar particle event exposures in deep space. *Radiat. Res.* 1999; 30:361–382. [PubMed: 11543148]
21. Smart DF, Shea MA. The local time dependence of the anisotropic solar cosmic ray flux. *Adv. Space Res.* 2003; 32:109–114. [PubMed: 14727670]
22. Kim MH, Hayat MJ, Feiveson AH, Cucinotta FA. Prediction of frequency and exposure level of solar particle events. *Health Phys.* 2009; 97:68–81. [PubMed: 19509510]
23. Hu S, Kim MH, McClellan GE, Cucinotta FA. Modeling the acute health effects of astronauts from exposure to large solar particle events. *Health Phys.* 2009; 96:465–476. [PubMed: 19276707]
24. Matalaka KZ, Tutunji MF, Abu-Baker M, Abu Baker Y. Measurement of protein cytokines in tissue extracts by enzyme-linked immunosorbent assays: application to lipopolysaccharide-induced differential milieu of cytokines. *Neuro. Endocrinol. Lett.* 2005; 26:231–236. [PubMed: 15990727]
25. Guarner F, Malagelada JR. Role of bacteria in experimental colitis. *Best Pract. Res. Clin. Gastroenterol.* 2003; 17:793–804. [PubMed: 14507589]
26. Vedantam G, Hecht DW. Antibiotics and anaerobes of gut origin. *Curr. Opin. Microbiol.* 2003; 6:457–461. [PubMed: 14572537]
27. Gruys E, Toussaint MJ, Niewold TA, Koopmans SJ. Acute phase reaction and acute phase proteins. *J. Zhejiang Univ. Sci. B.* 2005; 6:1045–1056. [PubMed: 16252337]
28. Jerala R. Structural biology of the LPS recognition. *Int J. Med. Microbiol.* 2007; 297:353–363. [PubMed: 17481951]
29. Kitchens RL, Thompson PA. Modulatory effects of sCD14 and LBP on LPS-host cell interactions. *J. Endotoxin Res.* 2005; 11:225–229. [PubMed: 16176659]
30. Haziot A, Rong GW, Lin XY, Silver J, Goyert SM. Recombinant soluble CD14 prevents mortality in mice treated with endotoxin (lipopolysaccharide). *J. Immunol.* 1995; 154:6529–6532. [PubMed: 7539025]
31. Estes JD, Harris LD, Klatt NR, Tabb B, Pittaluga S, Paiardini M, Barclay GR, Smedley J, Pung R, Brenchley JM. Damaged intestinal epithelial integrity linked to microbial translocation in pathogenic simian immunodeficiency virus infections. *PLoS Pathog.* 2010; 6:e1001052. [PubMed: 20808901]
32. Chatzantoni K, Mouzaki A. Anti-TNF-alpha antibody therapies in autoimmune diseases. *Curr. Top. Med. Chem.* 2006; 6:1707–1714. [PubMed: 17017952]
33. Gridley DS, Rizvi A, Luo-Owen X, Makinde AY, Coutrakon GB, Koss P, Slater JM, Pecaut MJ. Variable hematopoietic responses to acute photons, protons and simulated solar particle event protons. *In Vivo.* 2008; 22:159–169. [PubMed: 18468399]
34. Xie Y, Zhang H, Wang YL, Zhou QM, Qiu R, Yuan ZG, Zhou GM. Alterations of immune functions induced by $^{12}\text{C}^{6+}$ ion irradiation in mice. *Int. J. Radiat. Biol.* 2007; 83:577–581. [PubMed: 17654099]

35. Gridley DS, Dutta-Roy R, Andres ML, Nelson GA, Pecaut MJ. Acute effects of iron-particle radiation on immunity. Part II: Leukocyte activation, cytokines and adhesion. *Radiat. Res.* 2006; 165:78–87. [PubMed: 16392965]
36. Pecaut MJ, Gridley DS, Nelson GA. Long-term effects of low-dose proton radiation on immunity in mice: shielded vs. unshielded. *Aviat. Space Environ. Med.* 2003; 74:115–124. [PubMed: 12602442]
37. Balzan S, de Almeida Quadros C, de Cleve R, Zilberstein B, Cecconello I. Bacterial translocation: overview of mechanisms and clinical impact. *J. Gastroenterol. Hepatol.* 2007; 22:464–471. [PubMed: 17376034]
38. Sydora BC, MacFarlane SM, Lupicki M, Dmytrash AL, Dieleman LA, Fedorak RN. An imbalance in mucosal cytokine profile causes transient intestinal inflammation following an animal's first exposure to faecal bacteria and antigens. *Clin. Exp. Immunol.* 2010; 161:187–196. [PubMed: 20345974]
39. Hrnčir T, Stepankova R, Kozakova H, Hudcovic T, Tlaskalova-Hogenova H. Gut microbiota and lipopolysaccharide content of the diet influence development of regulatory T cells: studies in germ-free mice. *BMC Immunol.* 2008; 9:65. [PubMed: 18990206]
40. Packey CD, Ciorba MA. Microbial influences on the small intestinal response to radiation injury. *Curr. Opin. Gastroenterol.* 2010; 26:88–94. [PubMed: 20040865]
41. Hill GR, Crawford JM, Cooke KR, Brinson YS, Pan L, Ferrara JL. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. *Blood.* 1997; 90:3204–3213. [PubMed: 9376604]
42. Paulos CM, Wrzesinski C, Kaiser A, Hinrichs CS, Chieppa M, Cassard L, Palmer DC, Boni A, Muranski P, Restifo NP. Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling. *J. Clin. Invest.* 2007; 117:2197–2204. [PubMed: 17657310] *J. Clin. Invest.* 2007; 117:3140. Erratum.

FIG. 1.

Proton and γ radiation cause a breakdown in containment of gram-negative bacteria as measured by translocation of bacterial components across the GI tract and into the circulation. Mice were irradiated with 70 MeV protons (panel A) or γ radiation (panel B) at 50 cGy/h or 50 cGy/min. One day later, serum was analyzed for LPS content by a Factor C assay. Serum LPS levels were also measured 1, 5, 11 and 23 days after 2 Gy of 70 MeV protons at 50 cGy/h and in controls (panel C). Data shown are the averages for four mice per group analyzed in duplicate; error bars are SEM. Statistically significant differences between untreated and 2-Gy irradiated groups are indicated. Data shown are from a single experiment using 70 MeV protons on female mice and are representative of two experiments using male or female mice with 50 MeV or 70 MeV proton radiation.

FIG. 2.

Proton and γ radiation induce the acute-phase reactant LPS binding protein. Mice were irradiated with 70 MeV protons (panel A) or γ radiation (panel B) at 50 cGy/h or 50 cGy/min. One day later, serum was obtained and analyzed for LPS binding protein content by ELISA. Data shown are the averages for four mice per group run in duplicate, and error bars are SEM. Statistically significant differences between untreated and 2-Gy irradiated groups are indicated. Data shown are from a single experiment using 70 MeV protons on female mice and are representative of two experiments using male or female mice with 50 MeV or 70 MeV energy proton radiation.

FIG. 3.

Proton and γ radiation increase sCD14 levels. Mice were irradiated with 70 MeV protons at 50 cGy/h. One day later, serum was obtained and analyzed for sCD14 protein content by ELISA. Data shown are the averages for four mice per group run in duplicate; error bars are SEM. Statistically significant differences between the untreated and 2-Gy irradiated group are indicated. Data shown are from a single experiment using 70 MeV protons on female mice and are representative of two experiments using male or female mice with 50 MeV or 70 MeV energy proton radiation.

FIG. 4.

Proton and γ radiation induce a transient increase in circulating proinflammatory cytokines. Mice were irradiated with 2 Gy of 70 MeV protons and γ rays at 50 cGy/h (LDR) or 50 cGy/min (HDR). One day later, serum was obtained and analyzed for the proinflammatory cytokines TNF- α , IL-1 β and IL-6. Data shown are the averages for four mice per group run in duplicate; error bars are SEM. Statistically significant differences ($P < 0.05$) between irradiated and control groups are indicated by asterisks. Data shown are from a single experiment using 70 MeV protons on female mice and are representative of two experiments using male or female mice with 50 MeV or 70 MeV energy proton radiation.

FIG. 5.

Proton radiation induces breaks in the GI epithelial barrier. Terminal ileum obtained 2 days postirradiation from control and irradiated animals was stained for Claudin-3. Panel A: Ileum from mouse irradiated with 2 Gy low-dose-rate protons obtained 2 days after irradiation. Black arrows show regions of tight junction incongruity. Original magnification 400 $\times$. Panel B: Ileum from a control mouse stained for Claudin-3. Original magnification 400 $\times$. Panel C: Ileum from mouse irradiated with 2 Gy low-dose-rate protons stained with control rabbit Ig. Original magnification 400 $\times$. Ileum from five animals was analyzed for each treatment.

TABLE 1

Quantification of Claudin-3 Breaks in Terminal Ileum

Treatment	Breaks per 1000 enterocytes	Goblet cells per 1000 enterocytes	<i>P</i> value for breaks compared to control	<i>P</i> value for goblet cells compared to control
Control	0.06	21	–	–
2 Gy protons HDR ^a	0.71	35	0.028	>0.05
2 Gy protons LDR	0.86	42	0.034	>0.05
2 Gy γ rays, HDR	1.12	33	0.021	>0.05
2 Gy γ rays, LDR	0.85	39	0.041	>0.05

^aHDR, high dose rate; LDR, low dose rate.