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Biological Effects of Space Radiation and Development of Effective Countermeasures

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Abstract

As part of a program to assess the adverse biological effects expected from astronaut exposure to space radiation, numerous different biological effects relating to astronaut health have been evaluated. There has been major focus recently on the assessment of risks related to exposure to solar particle event (SPE) radiation. The effects related to various types of space radiation exposure that have been evaluated are: gene expression changes (primarily associated with programmed cell death and extracellular matrix (ECM) remodeling), oxidative stress, gastrointestinal tract bacterial translocation and immune system activation, peripheral hematopoietic cell counts, emesis, blood coagulation, skin, behavior/fatigue (including social exploration, submaximal exercise treadmill and spontaneous locomotor activity), heart functions, alterations in biological endpoints related to astronaut vision problems (lumbar puncture/ intracranial pressure, ocular ultrasound and histopathology studies), and survival, as well as long-term effects such as cancer and cataract development. A number of different countermeasures have been identified that can potentially mitigate or prevent the adverse biological effects resulting from exposure to space radiation.

1. Introduction

As reviewed by Hellweg and Baumstark-Khan (1), the primary components of radiation in interplanetary space are galactic cosmic rays (GCR) and solar cosmic radiation (SCR). GCR originates from outside of our Solar System and consists of 98% baryons and 2% electrons. The baryonic component consists of 87% protons (hydrogen nuclei), 12% alpha particles (helium nuclei) and approximately 1% of heavier nuclei with atomic numbers up to 92 (uranium). These heavier nuclei include highly energetic, heavy, charged particles known as HZE particles. Although ^{56}Fe ions, as a specific type of HZE particle, account for less than 1% of the GCR particle fluxes, ^{56}Fe ions contribute significantly to the total radiation dose received by individual cells exposed to GCR due to the fact that the dose to an individual cell is proportional to the square of the particle's energy dependent effective charge (2).

SCR consists of low energy solar wind particles that flow constantly from the Sun and the highly energetic solar particle events (SPEs) that originate from magnetically disturbed regions of the Sun, which sporadically emit bursts of energetic charged particles (3, 4). SCR is composed predominately of protons, with a minor contribution from helium ions (~10%) and an even smaller contribution from heavy ions and electrons (~1%). SPEs are unpredictable, develop rapidly and usually last for no more than several hours, although some SPEs may continue for several days. Since protons are the major component of SPE radiation, ground-based SPE radiation research is focused on the biological consequences of proton radiation at the appropriate energies, doses, and dose-rates expected during an SPE. A large fraction of the protons during a SPE are in the range of around 50 MeV, but there are also varying levels of protons of higher energies characterizing each individual SPE (5, 6).

Exposure to space radiation may place astronauts at significant risk for acute radiation sickness (ARS), significant skin injury and numerous other biological effects resulting from exposure to radiation from a major SPE, which normally includes some HZE particles, or combined SPE and GCR. Doses absorbed by tissues vary for different SPEs and model systems have been developed to calculate the radiation doses that could have been received by astronauts during previous SPEs (7). For instance, it has been estimated that the August 1972 SPE could have delivered doses of approximately 2.69 Gy and 0.46 Gy to skin and blood forming organs (BFO), respectively, in a spacecraft and 32 Gy and 1.38 Gy to skin and BFO, respectively, during extra-vehicular activity (EVA). Depending on the radiation dose, dose rate and quality, exposure to radiation during space missions may immediately affect the probability for successful mission completion (mission critical) or result in late radiation effects in individual astronauts (1). While avoidance of the radiation risk is the best protective strategy, it is nearly impossible to avoid the radiation risk completely for astronauts. Therefore, countermeasures against adverse biological effects of space radiation are necessary for the success of long term space missions. National Aeronautics and Space Administration (NASA) is primarily concerned with the health risks for astronaut exposures to GCR and SPE radiation. SPEs occur with variable tissue dose-rates and doses, which range from 0 to 0.5 Gy/hour and 0 to 2 Gy, respectively, and with skin doses > 5 Gy (7). NASA has determined that the likelihood of acute risks during internal vehicle activity is extremely small; however, there are scenarios during lunar, trans-lunar or Mars EVAs in which ARS may occur.

Acute radiation sickness has a sequence of a phased syndrome that varies with radiation dose, dose rate, quality and individual radiation sensitivity (1), which can include nausea, vomiting, diarrhea and fatigue. These effects are manifested at approximately 4 to 24 hours post-exposure for exposures at sub-lethal doses, with a latency time inversely correlated with dose. Since exposure to proton radiation, which represents the major type of radiation in an SPE, is known to induce abnormalities in leukocytes, erythrocytes and platelets (8), there is also a reasonable concern for compromised immune functions, especially in the microgravity environment in space (9–12). Space flight is known to alter immune responses, and the causal factors include the stress due to increased radiation exposure (13–15) and microgravity and non-load bearing status (16–19). The consistent effects on the immune system observed so far during space travel are as follows: reduction in peripheral T-cell

counts and a decrease in Natural Killer (NK) cell number and functionality (10, 20), decreases in cell-mediated immunity with altered cytokine production (21, 22) but normal levels of serum immunoglobulins (20). An increased susceptibility to infection under space flight conditions has also been observed (22, 23). The main concern of an impaired immune system in the closed environment of a spacecraft is the altered ability to control bacterial, fungal, viral, and parasitic invasions (10, 13, 20, 23) and the loss of immunosurveillance leading to tumor growth (24). Countermeasures that have been considered and/or evaluated for mitigating acute radiation effects on immune system include interferons, which have a profound effect on the immune response both *in vivo* and *in vitro* (25), an active hexose correlated compound, which activates immune function (26) and enhances resistance to infection (22, 27), and vitamin and mineral dietary supplementation, as recently reviewed (28).

In addition to acute effects from radiation, there are numerous other major health concerns related to space radiation exposure. In the NASA Human Research Roadmap (A Risk Reduction Strategy for Human Space Exploration, the Integrated Research Plan (IRP) divides the space radiation risks into the following categories: Risk of Acute and Late Central Nervous System Effects from Radiation Exposure, Risk of Acute Radiation Syndromes Due to Solar Particle Events (SPEs), Risk of Degenerative Tissue or other Health Effects from Radiation Exposure, and Risk of Radiation Carcinogenesis. The Degenerative Tissue Risks include adverse radiation biologic effects on the heart, circulatory, endocrine, digestive, lens and other tissue systems (which would include radiation effects on bone, muscle, etc.). It is noteworthy that the International Commission of Radiation Protection (ICRP) has recently issued a report that has important implications for two of the degenerative tissue risks, circulatory diseases and cataracts (29). In this recent review of early and late effects of radiation in normal tissues and organs, it was concluded that for reactions manifesting very late after low total doses, particularly for cataracts and circulatory disease, it appears that the rate of dose delivery does not modify the incidence, and for these two tissues, a threshold dose of 0.5 Gy was proposed (29). For a NASA mission to Mars, fatal cancer risk has been considered the dominant risk in the past (considering the dose from GCR), but circulatory diseases are likely to be of great importance in the newer risk estimates for a mission to Mars (30). There have been a number of recent reviews or updates on the status of space radiation research in the research areas of particular concern to NASA for the exploration class missions planned for the future.

For long term space exploration, bone loss and muscle atrophy due to disuse are other major concerns related to the health of the crew (31, 32). In ground-based studies, disuse bone loss has been observed in the hindlimb suspension rodent model (33–36). Irradiation with γ-rays exacerbates skeletal microarchitectural changes that are normally found during progressive, postpubertal aging prior to the onset of age-related osteoporosis (37). Radiation exposure may increase the number of osteoclasts and the extent of acute bone loss via increased reactive oxygen species production and oxidative damage, which implies different molecular mechanisms from the bone loss caused by disuse (38). Irradiation with 250 MeV protons followed by hindlimb suspension resulted in an approximately 20% loss of trabecular bone

volume fraction in the tibia and femur of irradiated mice, and the mice receiving the combined treatment with proton radiation and hindlimb suspension generally experienced greater loss of the trabecular bone volume fraction, connectivity density, and trabecular number than either hindlimb suspension or irradiation treatment alone (39). Irradiation with ^{56}Fe ions, which represents a significant component of GCR (2), stimulates osteoclast differentiation even in the absence of osteoblasts, thereby enhancing the sensitivity of bone cells to the effects of radiation (40). Iron ion radiation contributes to a reduction in compressive strength and partially prevents the recovery of cancellous microarchitecture from adaptive responses of lumbar vertebrae to skeletal unloading in hindlimb suspended mice (41). Thus, irradiation with heavy ions may accelerate or worsen the loss of skeletal integrity triggered by musculoskeletal disuse in the microgravity environment. There are some publications indicating that countermeasures may be helpful to mitigate radiation induced adverse bone effects, for example, α-lipoic acid protects cancellous tissue from the detrimental effects of irradiation (38).

The space radiation risks to central nerve system (CNS) have been considered to be extremely important in the recent past due to major publications in this field of research. Examples of such publications include studies suggesting that the induction of Alzheimer's disease may be a space radiation risk (42) and attention deficits may arise following exposure to low doses of space radiation (43). Many other publications have also indicated that there are major CNS space radiation risks [e.g., (44–52)]. Radiation exposure to γ-rays (53) or ^{56}Fe ions (54–57) is also known to have adverse effects on CNS and neurobehavior of irradiated animals, including reduced performance in motor tasks and deficits in spatial learning and memory. Alterations in neuronal function in the HZE particle irradiated animals include reduced responsiveness to agonist stimulation and increased Nigral cell loss, which parallel the neurobehavioral changes associated with aging (58, 59). The available data suggest that: a) the neurochemical and behavioral deficits after HZE radiation exposure have an apparent threshold below which there are no effects on these endpoints, b) there does not appear to be a dose-response curve for many endpoints, such as upper body strength or radiation induced taste aversion learning, and c) there is no evidence of spontaneous recovery of function that depends upon the integrity of the dopaminergic system after the HZE radiation exposure (60). It has been reported that persistent radiation induced oxidative stress is associated with space radiation induced CNS effects [e.g., (52)]. In the CNS research area, there have been several publications indicating that countermeasures exist for some of the space radiation induced adverse biological effects, which include melatonin or a metabolite (46), lipoic acid (47, 52), fruit extracts, which ameliorate deficits in behavior and signaling in rats irradiated with ^{56}Fe ions (57), and flavonoid glycosides from Ginkgo biloba, myricetin and quercetin (EGb761) (61), which have been postulated to improve cerebral metabolism, protect the brain against hypoxic damage and scavenge free-radicals (58).

Carcinogenesis has continued to be the major focus of the NASA space radiation risk experimental investigations over the past several years, with most of the investigations not focused on the development of tumors in animals developing from space radiation exposure(s), but instead focusing on various potential surrogate endpoint biomarkers (SEBs) of space radiation carcinogenesis. There are some recent reviews that focus on the

development of cancer in animals exposed to space radiation [e.g, (28, 62–65)], some recent individual reports on space radiation induced tumorigenesis [e.g., (65–68)], and some new hypotheses/thoughts concerning mechanisms of radiation carcinogenesis [e.g., (69, 70)] and risk estimates of radiation induced cancer [e.g., (71, 72)]. A notable finding in the recent animal carcinogenesis studies is that ^{56}Fe ions were not substantially more effective than γ-rays for the induction of acute myeloid leukemia (62, 65). It has been pointed out in numerous current and older reviews of space radiation carcinogenesis studies that space radiation induced malignancies are dependent on the species as well as the strain of the species used, and that a major task in this field of research will involve determinations about the appropriate methods to use for extrapolation of the space radiation induced cancer risks from experimental animal studies to humans. One example of the differences observed in space radiation induced cancer studies concerns the development of hepatocellular carcinoma. While exposure to space radiation(s) has indicated a very high incidence of hepatocellular carcinoma in one mouse strain (62, 65), in other experiments on space radiation induced carcinogenesis using a different strain of mice, a dose of 0.5 Gy from ^{56}Fe ions or 3 Gy from protons had no effects on the development of hepatocellular carcinoma (73). There are some intriguing recent results in the radiation carcinogenesis field of research. Ding et al. have indicated that there are distinct signatures (transcriptome profiles) in normal human bronchial epithelial cells exposed to γ-rays and different HZE particles (74). If this effect can be confirmed, it may give rise to studies in which the causative agent can be identified in human malignancies that could have been caused by radiation exposure. While the mechanism(s) involved in space radiation induced carcinogenesis are still unknown, there is evidence that space radiation induced oxidative stress is closely associated with carcinogenesis [e.g., (28)]. It has been reported that space radiation induces persistent oxidative stress in mouse intestine, which is likely to be associated with intestinal tumorigenesis (75). There is evidence that space radiation induced carcinogenesis can be prevented or mitigated by several cancer chemopreventive agents, which include antioxidants and protease inhibitors [as recently reviewed (28)], retinoids [e.g. (76–78)] and fruit extracts (79, 80). In addition, there are a number of new potential cancer preventive agents that have been shown to mitigate *in vitro* SEBs of the space radiation cancer risk; examples include melatonin (81) and a synthetic triterpenoid, bardoxolone methyl, which protects against space radiation-induced transformation of human colon epithelial cells (82).

There is extensive evidence that radiation exposure on earth can give rise to cardiovascular diseases, as recently reviewed (29, 83–85). In the research on heart and circulatory effects resulting from exposure to space radiation(s), it has recently been reported that doses of 2 to 5 Gy ^{56}Fe ion radiation targeted to specific arterial sites in apolipoprotein E-deficient (apoE$^{-/-}$) mice accelerated the development of atherosclerosis (86). In these studies, it was concluded that ^{56}Fe ions can promote the progression of atherosclerotic lesions to an advanced stage characterized by compositional changes indicative of increased thrombogenicity and instability. In numerous other studies, space radiation has been shown to have detrimental effects of many other parameters related to cardiovascular and circulatory diseases, with particularly strong effects leading to endothelial dysfunction [e.g., (87)] and angiogenesis [e.g., (88–91)].

Risks of other degenerative diseases include radiation induced cataracts, as recently reviewed (29, 92–97). It is noteworthy that the ICRP has recently proposed lowering the threshold for radiation induced cataracts to 0.5 Gy (29). There have also been some recent studies on space radiation (or other similar types of radiation such as heavy ion-cancer therapy [hadron therapy]) induced cataracts (98–102). It has been reported that astronauts have an elevated risk of developing cataracts (103, 104), which has been associated with exposure to the high linear energy transfer (LET) GCR present in the space environment.

Current medical treatment for the acute radiation syndrome routinely includes supportive care, antibiotics (quinolones and other agents), cytokine therapy, antiemetic agents and analgesic agents (105, 106). Other agents can also be used for the effects of the acute radiation syndrome, such as antihistamines, anti-inflammatories and radioprotectors (107). Several FDA approved anti-emetic drugs, such as Kytril (granisetron), Zofran (Ondansetron), Decadron® (dexamethasone tablets) and Emend (Aprepitant) are known to prevent or alleviate nausea and vomiting in patients or animals exposed to radiation or chemotherapeutic agents (108–111). A systematic review and meta-analysis of 14 randomized controlled trials, comprising 1451 patients, shows that amifostine (WR2721) significantly reduces the side effects of radiation therapy (112). In the animal studies, treatment with amifostine was shown to protect against DNA damage in cisplatin treated murine peripheral leukocytes (113), reduce changes in nucleolar morphology induced by cisplatin treatment (114) and protect against cyclophosphamide-induced disruption of taste (115) and methotrexate-induced small intestinal mucositis (116), as well as inhibit tumorigenesis (117). Unfortunately, the severe side effects of amifostine have limited its use in the space program for astronauts as well as in other human populations exposed to radiation. PrC-210 is a new aminothiol that has shown no detectable nausea/vomiting or hypotension side effects in the ferret and rat models (118), in contrast to the strong side effects of the current aminothiol, amifostine (119, 120); this compound shows promise as a new aminothiol radioprotector.

In the past several years, we have been engaged in research to assess whether there will be adverse acute biological effects similar to those of ARS after exposure to the types of radiation at the energies, doses and dose-rates expected during an SPE. The overall objectives of our studies were to assess the risk of ARS and evaluate countermeasures for ARS, which can develop after exposure to SPE radiation. There is also a reasonable concern for a compromised immune system, due to high skin doses from an SPE, which can lead to burns. Existing evidence suggests that the best animal model for radiation induced vomiting is the ferret (121, 122) whereas the best animal model for radiation induced skin changes is the pig (123–126). Mice, on the other hand, are the most frequently utilized mammalian species for evaluation of many radiation induced biologic effects. A major problem concerning the use of mice for studies of SPE radiation is that mice do not vomit in response to irradiation (127). Therefore, three species of animals, i.e., ferrets, pigs and mice, were used in our studies to allow interspecies comparisons of the results obtained in the studies in several different areas of research, whereas the effects on vomiting and skin were evaluated only in the most appropriate animal species. Since astronauts will be exposed to space radiation in a microgravity environment, which is known to cause bone loss, muscle atrophy and injury to soft connective tissues in animal models (31, 33), some of the radiation

experiments with mice have been performed with and without partial weight suspension (PWS) at one-sixth G, which is known to be the gravity on the surface of the Moon (128), or hindlimb suspension (HS), a model appropriate for mimicking travel in deep space (36) to evaluate and quantify the possible synergy between radiation and simulated hypogravity on hematopoietic effects associated with ARS.

In the studies in which SPE radiation effects have been evaluated, it was considered extremely important to have comparable dose distributions between the SPE radiation and a standard reference radiation in the animal model systems so that relative biological effectiveness (RBE) values could be calculated (129). SPE radiation is known to result in inhomogeneous total body distribution, with a considerably higher dose delivered to the skin and underlying tissues than to the internal organs (7). These characteristics of an SPE, which result in an unusual dose distribution pattern, make it difficult to compare the results of SPE radiation with conventional γ-ray radiation. The dose distribution from electron radiation, however, can be manipulated to simulate is the dose distribution expected from SPE radiation. Megavoltage electron beam radiation has been utilized in the pig experiments to accurately reproduce the total dose and dose distribution of SPE protons (129). The dosimetry involved in determining simulated SPE radiation doses in pigs is illustrated in Figure 1, which shows that the doses to the external organs (e.g., skin, eyes) are very high, while the doses to internal organs (e.g., spinal cord, bone marrow) are quite low. The detailed methods for determining the organ doses are described elsewhere (130, 131). These methods incorporate modern radiation oncology approaches of computed tomography (CT) based Monte Carlo dosimetry into the studies so that acute biological effects in specific organ systems can be determined in animal model systems, and radiation toxicity from various types of SPE radiation exposures can be compared. This approach has also been used to predict the acute biological effects of SPE radiation exposure in astronauts. Ten full body human CT scans in various geometries have been analyzed to determine the impact of physical and environmental factors on organ dosimetry in humans. It has been found that, depending on the organ system of interest (deep vs. superficial) and the fluence/energy profile of the exposure (hard vs. soft event), either the physical size of the astronaut or the fluence/energy profile for the SPE can be the determining factor for radiation dose/toxicity (Cengel, K.A., Schaettler, M.O., and Diffenderfer, E., Unpublished data). In contrast, most of the experiments with mice or ferrets utilizing SPE-like proton radiation involved homogeneous proton radiation exposures with relatively low energies like those present in SPEs; RBE values were then calculated by comparison of the SPE-like proton results with those obtained in similar experiments using γ-ray radiation.

In this review paper, the results of our studies on the biological effects of several different types of space radiation, which include different types of SPE radiation, are discussed. Both acute and chronic effects of space radiation have been evaluated in these studies.

2. Acute Radiation Effects

The acute radiation effects evaluated in our studies include effects on hematopoietic cells, immune system effects (which include immune system changes resulting from a high dose

of SPE radiation to the skin), behavior/fatigue, heart functions, skin effects and organism survival after exposure to a lethal or potentially lethal dose of radiation.

2.1. Changes in Hematopoietic Cell Counts after Proton or Conventional Reference Radiation Exposures

The changes in peripheral leukocytes in animals post-irradiation have been evaluated in ICR mice irradiated with 225-kVp X-rays (132), γ-rays from ^{60}Co (133) or ^{137}Cs (134–136), protons with energies of 50-MeV (133), 51-MeV (137), 70-MeV (133), 74-MeV (134, 135), 78.4 MeV (138) and 1-GeV protons (137, 139) as well as protons with eight different energies between 31 and 75 MeV and simulated SPE protons with energies between 30 and 150 MeV (140). The changes in peripheral leukocytes have also been examined in ferrets irradiated with ^{60}Co or ^{137}Cs γ-rays and 110-MeV protons (141, 142) and Yucatan minipigs irradiated with 6-MeV electrons (130), 6+12-MeV electrons and SPE protons (143, 144).

2.1.1. Changes in Hematopoietic Counts in Mice after Irradiation—In an early study focused on determining the changes in circulating hematopoietic cell counts in mice exposed to SPE-like proton or γ-ray radiation (used as the reference radiation), ICR outbred mice aged 5–6 weeks were exposed to ^{60}Co γ-rays at doses of 0.13, 0.25, 0.5, 1 or 2 Gy or spread out Bragg peak (SOBP) protons (50 or 70 MeV) at doses of 0.25, 0.5, 1 and 2 Gy. The radiation exposures were delivered in a single dose at the low dose rate of 0.5 Gy/hour or the high dose rate of 0.5 Gy/minute. The results demonstrated a dose dependent decrease in white blood cell (WBC) counts in mice exposed to high and low dose rate proton and γ-radiation (133).

In 4–5 week old ICR mice, peripheral WBC, lymphocyte and/or polymorphonuclear leukocyte (PMN) counts decreased significantly at 4 and 24 hours after total body irradiation with 1 or 8 Gy of 225 kVp X-rays (132) or 1 or 5.9, 6.8 or 7.2 Gy of 1 GeV protons (139). At 24 hours after irradiation with 8 Gy of 225 kVp X-rays, the neutrophil count was decreased to an average of 450 cells/μl; neutrophil counts of < 500 cells/ μl are clinically significant. At 8 weeks post-irradiation, peripheral WBC and lymphocyte counts in the mice irradiated with 8 Gy of X-rays were still 64% and 76% below the respective control values (132), whereas peripheral WBC and lymphocyte counts in the mice irradiated with 5.9 Gy of 1-GeV protons were still approximately 37% and 44% below the respective control values in non-irradiated control animals (139). In contrast, the PMN/neutrophil count was fully recovered by 8 weeks post-irradiation with 8 Gy of 225 kVp X-rays (132).

In a separate study performed with male ICR mice aged 4–5 weeks, exposure to 1 GeV proton radiation administered in a single dose at low (5 cGy/minute) or high (50 cGy/minute) dose rates, or in five fractionated doses at the low dose rate resulted in significant and dose dependent decreases in peripheral WBC and lymphocyte counts at 24 hours post-irradiation (137). However, the difference among animals irradiated with the five fractionated doses, or in a single dose at low or high dose rate, did not reach statistical significance at any of the doses evaluated, and neither the WBC counts nor the lymphocyte counts in the animals irradiated with 2 Gy 1-GeV protons in the five fractionated doses, or in a single dose at low or high dose rate, were significantly different from the animals

irradiated with 2 Gy of 51.24-MeV protons administered at a low dose rate (5–7 cGy/minute), although they were all significantly below the control WBC and lymphocyte counts in sham irradiated animals. These results suggest that the effect of proton irradiation on the WBC and lymphocyte counts in the irradiated mice is not altered by dose fractionation, dose rates or proton energy in the ranges evaluated (137).

Some experiments were performed to determine whether age or sex/gender differences affected the ability of SPE-like radiation to affect circulating hematopoietic cell counts in mice. To determine the effects of age on this endpoint, 1-year old ICR mice were exposed to proton or γ-ray radiation at doses of 0.0, 0.5, 1.0, or 2.0 Gy, with a dose rate of 0.5 Gy/min (133, 145). Whole blood samples were collected up to 30 days post- irradiation and complete blood counts were analyzed using automated technology as previously described (133, 145, 146). A comparable experiment was performed in young ICR mice, aged 6–8 weeks. Statistical analyses and RBE values were determined as previously described (145) for the experiments performed in both young and aged mice. The older mice in this experiment were specifically aged to simulate healthy, non-smoking, middle-aged astronauts. For example, the preferred age range for European Space Agency applicants is 27–37 years old. The decline in WBCs, neutrophils, lymphocytes and granulocytes were not different using aged mice as compared to the reduction observed in young mice (Sanzari, J.K. and Kennedy, A.R., Unpublished data). The lymphocyte nadir was at 2 days post-proton irradiation, with counts as low as 50%, 60%, and 77% of the control values after exposure to 0.5, 1.0 and 2.0 Gy protons, respectively. The decline in lymphocyte counts after γ-ray radiation exposure was similar to that observed after the proton radiation exposure, which were also comparable to the decline observed in young mice. The results for the granulocyte counts were also consistent between the aged and young mice, with a bimodal decline observed for the first nadir at 4 days post-irradiation (γ-ray or proton) and the second nadir at 16 days post-irradiation. The RBE values at different time points and for each dose were calculated in the aged and young mice. It was observed that the RBE values were not significantly different from 1.0 in either the young or the aged mice. In similar experiments, the radiation effects on WBCs, lymphocytes and granulocytes were shown to be similar between male and female CHH/HeN mice (147). The only sex/gender difference observed in these experiments was that non-irradiated male mice had 13% higher platelet counts and more enhanced recovery of platelets on day 16 after irradiation as compared to female mice. Thus, it is conceivable that this difference between male and female mice could influence the response of platelets to total body radiation exposure.

In a study conducted with 5 to 7-week old female ICR mice, exposure to protons with 8 energy levels ranging from 30.63 to 74.52 MeV or simulated SPE protons with energies ranging from 30 to 150 MeV at high (0.5 Gy/minute) or low (0.5 Gy/hour) dose rate resulted in significant decreases in peripheral WBC and lymphocyte counts as early as 4 hours post-irradiation (140). At 24 hours post-irradiation with the 30.63–74.52 MeV protons, the dose response curves were nearly identical between the mice irradiated at the low and high dose rates for WBCs or lymphocytes. In a separate experiment performed in the same series of experiments, the mice were irradiated with protons at a very low dose rate of 0.28 cGy/minute (17 cGy/hour), and the results were similar to those of the mice irradiated at the high dose rate (140). These results again indicate that dose rate in the range evaluated has little or

no impact on the suppressive effects of proton radiation on the peripheral WBC or lymphocyte counts in irradiated mice.

During space missions, astronauts are potentially exposed to SPE radiation in a reduced gravity environment. Thus, several experiments have been performed to determine the effects of simulated microgravity on blood cell counts with or without additional exposure to space radiation [e.g., (148)]. To evaluate the impact of hypogravity on the effect of SPE radiation on immunological function, experiments were performed with 6–8 week old female ICR mice that were irradiated with 0.5, 1 or 2 Gy of γ-rays with or without hypogravity simulated using the PWS model, described by Wagner et al. (149). The combination treatment with PWS and γ-ray irradiation decreased total splenic lymphocyte viability in a dose dependent manner, and the suppressed splenic lymphocyte viability in groups exposed to a 2 Gy dose of radiation persisted for 4 days, which was the last time point evaluated in the study (136). In addition, the viability of splenic lymphocytes was significantly lower in the mice that received a 1 Gy dose of γ-rays in combination of PWS treatment than in the mice that received a 1 Gy dose of γ-rays without PWS treatment on Day-1 or Day 4 post-irradiation. Treatment with PWS alone did not significantly affect the splenic lymphocyte viability at any of the time points evaluated up to 4 days. These results suggest that simulated hypogravity might have made splenic lymphocytes more sensitive to the cell killing effects of radiation. In addition, results from these studies indicated that T cell activation was decreased in the irradiated mice (1 or 2 Gy) with or without simulated hypogravity (PWS). Similar results were observed in experiments using mice exposed to SPE-like proton radiation with and without simulated hypogravity produced by HS (134, 138). In these experiments, mice were suspended prior to and after SPE proton radiation exposure and total leukocyte numbers and splenic lymphocyte functions were evaluated on days 4 or 21 after the radiation exposure with and without HS. Splenic lymphocyte subpopulations were altered at both time points investigated. At 21 days post-exposure, T cell activation and proliferation were assessed in isolated lymphocytes. In these studies, T cell activation was suppressed in the proton-irradiated animals and in the irradiated animals exposed to HS. From both types of experiments described above, the results suggest that these irradiated animals with or without additional exposure to simulated microgravity would have immune system suppression resulting from the lack of T cell activation. However, the peripheral blood cell (lymphocyte and granulocyte) counts were significantly higher in proton irradiated mice with HU treatment than without HU treatment and the HU treatment did not significantly interact with the proton radiation dose in the blood cell count data, indicating that the effects of radiation and hypogravity on peripheral leukocytes were simply additive (or subtractive) with no significant synergy (134).

To determine the RBE values for the effects of SPE protons in hematopoietic cells, several studies were performed in mice using 21-MeV electrons (150), ^{60}Co γ-rays (133) or ^{137}Cs γ-rays (135, 145) as the reference radiations. In male ICR mice irradiated with 2 Gy of 70-MeV protons or 21-MeV electrons, peripheral WBC, lymphocyte and granulocyte counts decreased significantly in the irradiated mice as compared to the sham irradiated control, but the differences between the proton and electron irradiated mice were not statistically significant for WBCs, lymphocytes or granulocytes (150). In 5 to 6-week old female ICR mice irradiated with ^{60}Co γ-rays, 50-MeV protons or 70-MeV protons at low (0.5 Gy/hour)

or high (0.5 Gy/minute) dose rate, the peripheral WBC count decreased in a dose-dependent manner at 24 hours post-irradiation and the RBE values for 50-MeV and 70-MeV protons at either low or high dose rate were not significantly different from 1 with ^{60}Co γ-rays as the reference radiation at the corresponding dose rates (133). In a separate study performed with 6-week old female ICR mice, the neutrophil count was monitored for 30 days post-irradiation with 0.5, 1 or 2 Gy of ^{137}Cs γ-rays or 74-MeV protons at dose rates of 0.44 Gy/minute and 0.5 Gy/minute, respectively (135). For the 2 Gy γ-ray and proton dose groups, the neutrophil counts decreased by approximately 65% and 70%, respectively, at the first nadir, which occurred on Day-4 post-irradiation, and then fully recovered by Day-10 post-irradiation to levels that were not significantly different from the pre-irradiation control level. The neutrophil counts for the 2 Gy γ-ray and proton dose groups decreased again by approximately 80% and 75%, respectively, at the second nadir, which occurred by Day-16 post irradiation, and then fully recovered again by Day 23 post-irradiation. Comparable results were observed in similar studies utilizing 74-MeV protons and ^{137}Cs γ-rays in which the changes in WBC counts were evaluated over time (145). The results from the studies in mice indicate that proton radiation is not more effective than the commonly used reference radiations with respect to effects on hematopoietic cells (135, 145).

Overall, the results show that the RBE values are not significantly different from 1 when the effects of SPE-like protons are compared to those from the reference radiations (γ-ray or electron radiation) in mice, and that SPE-like protons and γ-ray radiation result in almost identical dose-response curves over time, as illustrated in Figure 2 [Data in this figure are from (145)].

2.1.2. Changes in Peripheral Leukocytes Counts in Ferrets after Irradiation—In ferrets irradiated with up to 2 Gy of ^{60}Co γ-rays or 110-MeV protons at a high dose rate (HDR) of 0.5 Gy/minute or a low dose rate (LDR) of 0.5 Gy/hour, the white blood cell (WBC) count decreased significantly within 3 hours after the radiation exposure, and the average magnitude of the WBC decrease in the groups irradiated with 2 Gy of γ-ray or proton radiation at 48 hours post-irradiation was approximately three times the decrease observed at 3 hours post-irradiation (141). The lymphocyte count also decreased significantly within 3 hours after irradiation with ^{60}Co γ-rays or 110-MeV protons, but the magnitude of the decrease was similar at the two time points (3 and 48 hours) post-irradiation. In contrast, the neutrophil count increased significantly at 3 hours post-irradiation ($p < 0.001$) and then decreased significantly at 48 hours post- irradiation ($p < 0.001$), both in a dose-dependent manner.

In addition to the radiation dose, the dose rate also affected the WBC and neutrophil counts at 3 hours, but not at 48 hours post-irradiation, when the WBC and neutrophil counts in the groups irradiated at HDR were lower by approximately 16% and 32%, respectively, as compared to the WBC and neutrophil counts in the groups irradiated at LDR (141). The dose rate effects for the WBC and neutrophil counts were small, as compared to the magnitude of the effect of radiation dose at 3 hours post-irradiation, and disappeared by 48 hours post-irradiation, suggesting that the higher dose rate might have only accelerated the onset of the radiation effects, but did not affect the overall magnitude of the radiation effects that developed at the later time points. Given the fact that the dose rate effect was not

observed at 48 hours post-irradiation, when more pronounced losses of circulating WBCs, neutrophils and lymphocytes were observed in the irradiated ferrets, the dose rate effect probably did not have a biologically meaningful impact on the blood cell counts in the irradiated animals.

RBE values were determined for 110 MeV protons using ^{60}Co γ-rays as a reference radiation and peripheral leukocyte counts in the irradiated ferrets as the biological endpoints. The RBE values derived from the WBC counts for 110 MeV protons delivered at the high or low dose rates at 3 and 48 hours post- irradiation ranged from 1.19 to 4.02 at 0.75 Gy and declined with the increase in the radiation dose to a narrow range of 0.59 to 1.04 at 2 Gy (141). The RBE values calculated from the lymphocyte count data for the 110 MeV protons at the same time points and dose rates were within a range of 0.83 to 1.41 at 0.75 Gy and showed a slightly downward trend with the increase in the proton radiation dose to a narrow range of 0.67 to 0.84 at 2 Gy. With only a few exceptions, a similar downward trend was also observed for the RBEs based on the neutrophil, monocyte and eosinophil counts in the irradiated ferrets. These results suggest that 110-MeV protons might be more effective than ^{60}Co γ-rays in reducing the peripheral leukocyte counts at the low end of the radiation dose range evaluated in ferrets.

While the early radiation induced changes in the blood cells of ferrets were similar to those in the irradiated mice, the later portion of the time course was quite different between mice and ferrets. In the irradiated mice, the initial sharp decrease in WBC counts post-irradiation is followed by a gradual recovery to baseline levels by 30 days post-irradiation. However, the recovery in the WBC counts did not occur in ferrets exposed to protons at doses of 1.5 Gy and higher (involving ferrets exposed to a 2 Gy dose of proton radiation). This was shown to be due to the development of disseminated intravascular coagulation (142), as discussed below.

2.1.3. Changes in Peripheral Leukocyte Counts in Yucatan Minipigs after Irradiation—In the Yucatan minipigs irradiated with 6-MeV electrons at a total skin dose of 25 Gy, the WBC count decreased significantly on day-1 post-irradiation, and then recovered by day-7 and increased significantly above the pre-irradiation level by day-30 after irradiation (130). The WBC count did not change significantly in any other dose groups irradiated with electrons at a dose of 15 Gy or below. The lymphocyte count in the minipigs decreased significantly as early as 4 hours post- irradiation with 6-MeV electrons at skin doses of 15 or 25 Gy then recovered to the pre-irradiation level by day-14 and day-7 for the 15-Gy and 25-Gy dose groups, respectively. The neutrophil count did not change significantly after exposure to the electron irradiation in any dose groups at any time points except for a two-fold increase in the neutrophil count in the 25-Gy dose group at day-30 after irradiation.

In the Yucatan minipigs exposed to radiation with 6+12 MeV electrons, which is a suitable reference radiation with comparable body dose distribution as the SPE radiation (129), the WBC count decreased significantly within a day post- irradiation in the 10, 15 and 20 Gy dose groups, but not in the groups irradiated at a dose of 7.7 Gy or below (144). Between day-1 and day-7 post- irradiation, the WBC count reached the lowest level, and then

recovered slowly thereafter. By day-30, the WBC counts in the 10, 15 and 20 Gy dose groups all recovered to levels that were not significantly different from the baseline value. The significant decrease in lymphocyte counts occurred earlier, to a greater extent and extended to lower dose (i.e., 5, 7.5 and 7.7 Gy) groups than the decrease in the WBC count after the 6+12 MeV electron irradiation. Within a day after irradiation with 6+12 MeV electrons at doses up to 20 Gy, the lymphocyte count decreased by up to 77%. By day-30 post-irradiation, the lymphocyte count in the 5, 7.5, 7.7 and 15 Gy dose groups recovered to levels that were not significantly different from the baseline value; however, the lymphocyte counts in the 10 and 20 Gy dose groups were still significantly below the baseline level. The neutrophil count in the Yucatan minipigs irradiated with 6+12 MeV electrons did not show a consistent pattern of change among different dose groups post-irradiation. Both increases and decreases in the neutrophil counts were observed at different time points post-irradiation with 6+12 MeV electrons at skin doses up to 15 Gy, although the changes did not reach statistical significance due to the relatively large variations in the control group and in some of the irradiated groups.

In the Yucatan minipigs irradiated with SPE-like protons with energies of 155 MeV or below, the WBC count decreased significantly within a day post- irradiation with a single skin dose of up to 10 Gy (143). Between day-1 and day-4 post- irradiation, the WBC count reached the lowest level, and then recovered slowly thereafter. By day-30, the WBC count was no more than 38.3% below the baseline level for the animals irradiated with 5, 7.7 or 10 Gy doses of protons. By day-90, the WBC count recovered fully for the 5 Gy radiation dose group while remaining 18.7% and 33.5% below the baseline level for the 7.7 and 10 Gy radiation dose groups. Significant decreases in lymphocyte counts occurred earlier and to a greater extent than the decrease in the WBC count for the minipigs irradiated with 5, 7.7 and 10 Gy of protons. On day-1 after irradiation, the lymphocyte count reached the lowest level, which was 73.0%, 79.7% and 89.5% below the baseline level for the 5, 7.7 and 10 Gy radiation dose groups, respectively. By day-30 post- irradiation, the lymphocyte count in the irradiated animals was not more than 34.5% below the baseline level. By day-90, the lymphocyte count recovered fully for the 5 Gy radiation dose group and was not more than 19.5% below the baseline level for the two higher radiation dose groups.

The neutrophil count change in the Yucatan minipigs displayed quite a different time course as compared to the changes observed in the WBC and lymphocyte counts. The neutrophil count increased by up to 79.8% at 4 hours post- irradiation, and then decreased by up to 42.1% on day-1 after irradiation. The neutrophil count reached the lowest level between day-4 and day-14 post-irradiation, and then recovered slowly thereafter. By day-90 post-irradiation, the neutrophil counts in the 7.7 Gy and 10 Gy dose groups were still 29.3% and 48.0% below the pre-irradiation level. The results for the pig WBC counts over a 30 day experimental period following exposure to proton or electron radiation are shown in Figure 3. It should be noted that, following exposure to electron simulated SPE radiation, the WBC counts return to normal levels by 30 days post-irradiation, but the WBC counts in pigs exposed to proton simulated SPE radiation do not return to normal levels over this time period.

Based on the WBC, lymphocyte and neutrophil count data for the minipigs exposed to 6+12 MeV electron radiation (144) and simulated SPE radiation (143), RBE values were calculated for the effect of simulated SPE radiation on leukocytes in irradiated animals. The results show that the RBE value for the simulated SPE radiation varied with both the radiation dose and the time post-irradiation (144). For WBC counts, the RBE calculated for the simulated SPE radiation displayed a downward trend with the increase in radiation dose on Day-1, Day-4 and Day-14 post-irradiation. At the 5 Gy proton dose level, the RBE values for the simulated SPE radiation were 2.0, 4.1 and 3.3 on Day-1, Day-4 and Day-14, respectively, after irradiation (144). For lymphocyte counts at 4 hours post-irradiation, the RBE for the simulated SPE radiation also showed a downward trend with increasing dose, with the RBE value changing from 9.6 at 5 Gy to 4.6 at 10 Gy. However, the RBE trend for the simulated SPE radiation calculated from the lymphocyte count data was relatively flat on Day-1 and Day-4 and only slightly downward with the increase in the radiation dose on Day-14 post-irradiation. For neutrophils, the RBE for the simulated SPE radiation also displayed a noticeable downward trend with the increase in radiation dose on Day-4 and Day-14. The fitted RBE values were higher than 1.00 at all three simulated SPE radiation dose levels of 5, 7.7 and 10 Gy for WBCs and lymphocytes, and the lower limits of the 95% confidence interval for the RBEs were above 1.00 for all dose levels on Day-1, 4 and 14 except for 10 Gy on Day-1 for WBCs and 7.7 and 10 Gy on Day-4 for neutrophils. In addition, the ED_{10} and ED_{50} values for the simulated SPE proton radiation were significantly lower than those for the 6+12 MeV electron radiation on Day-1, 4 and/or 14 post-irradiation for WBCs, lymphocytes and/or neutrophils (144). These results indicate that simulated SPE proton radiation is significantly more effective than 6+12 MeV electrons with respect to the effects on the peripheral WBCs, lymphocytes and neutrophils, especially at the low end of the radiation dose range evaluated. It was observed that the neutrophils ware particularly sensitive to the proton damaging effects of proton radiation.

2.1.4. Summary of Effects of SPE Radiation on Hematopoietic Blood Cell Counts in Ferrets, Mice and Pigs—For hematopoietic cells, significant decreases in white blood cell counts were observed in mice and ferrets irradiated at high (~0.5 Gy/minute) and low (0.5 to 0.17 Gy/hour) dose rates, starting at doses of 0.5 Gy and up to 2 Gy. In some, but not all, of the studies involving dose-rate comparisons, lowering the dose rate had a small, but statistically significant, sparing effect on the white blood cell count parameters evaluated, but in many other dose-rate experiments performed, such changes were not observed. Thus, over the range of dose-rates evaluated, it is concluded that lowering the radiation dose-rate does not produce sparing effects on hematopoietic cell counts that are of biological significance. At the higher doses of proton or γ-ray radiation, the neutrophil counts in the blood of both mice and ferrets reach critically low levels (<500 cells per microliter) (135, 142, 145). If such a low value occurred in a patient in a hospital (e.g., following radiation or chemotherapy for cancer), this would trigger a medical response, and suggest with the use of countermeasures to increase the level of neutrophils. For the pigs, there were also highly significant reductions in the levels of WBCs following proton or electron SPE radiation exposure, but they did not reach the critically low values observed in the mice or ferrets at any of the high skin doses evaluated.

A major difference in the pig response to the electron and proton SPE radiation was that the neutrophil count did not show a meaningful recovery by 3 months after exposure to 10 Gy of proton SPE radiation (143). For pigs exposed to 10 or 20 Gy of electron SPE radiation, the neutrophil count recovered as expected within a month after the radiation exposure (143). These results indicate that the pigs might be less capable of repairing the DNA damage caused by the proton radiation exposure than the DNA damage caused by the electron radiation at similar or higher doses. Similar results were observed for mice exposed to proton total body radiation at a dose of 5.5 Gy, which significantly suppressed the neutrophil count at 9 weeks post-irradiation (139). In contrast, the neutrophil count in mice exposed to x-rays at comparable or considerably higher doses (up to 8 Gy) was fully recovered at 8 weeks post-irradiation (132). These results suggest that mouse neutrophils might also be more sensitive to the DNA damaging effects of proton radiation when given at a relatively high dose (5.5 Gy). At a dose of 2 Gy, the neutrophils in the irradiated mice appeared to be equally sensitive to the damage from proton and γ-ray irradiation (135, 145).

We have calculated RBE values for SPE-like radiations using hematopoietic cell count data from mice, ferrets and Yucatan minipigs irradiated with SPE-like proton radiation and a suitable reference radiation (e.g., γ-rays, x-rays or electrons). A higher RBE value for a given biological endpoint, such as blood cell count, indicates that the SPE-like proton radiation is more effective than the reference radiation in affecting that biological endpoint. In these studies, it was observed that there were: 1) different RBEs for different biological endpoints in the same animal species/strain, and 2) different RBEs for the same endpoint in different species/strains. The RBE values estimated based on the WBC results vary greatly between mice, ferrets and pigs, with the RBE values being greater in ferrets than in mice at times up to 48 hours post-irradiation (141), and considerably greater in pigs than in ferrets and mice (144). This trend suggests that the RBE values for WBC counts in humans could be considerably greater than those observed in smaller mammals, and SPE proton radiation may be far more hazardous to humans than previously estimated from studies performed with small animals.

2.2. Immune System Effects

2.2.1. Effect of SPE-like Radiation on Gastrointestinal Tract Integrity—

Numerous studies have been performed to evaluate the effects of SPE radiation on the immune system as a part of research on the acute risks of SPE radiation exposure (138, 144,148, 151, 152). Many of the SPE radiation studies were performed with and without microgravity simulated with PWS or HS (134, 138,148, 151–153). For the studies related to immune system effects, mice were exposed to homogeneous doses of either γ-ray or SPE-like proton radiation. The effects of γ-ray and SPE-like proton radiation were comparable in these studies, and none of the observed effects described below were specific to proton or γ-ray radiation.

The gastrointestinal (GI) tract contains over 10^{12} bacteria, and these bacteria have many important functions including carbohydrate fermentation and absorption, repression of pathogenic microbial growth, and continuous modulation of the gut and systemic immune system. A critical function of the GI tract is the containment of commensal bacteria, which

involves the control of bacteria and bacterial product passage across the GI mucosa, known as bacterial translocation; this function can be disturbed in many different diseases. In a study performed with ICR mice at 5–6 weeks of age, irradiation with 2 Gy of 50 or 70-MeV protons resulted in a transient increase of lipopolysaccharide (LPS) in the serum at one day post-irradiation, and the increase was accompanied by increases in acute-phase reactants, such as lipopolysaccharide binding protein (LBP) and soluble CD14 (sCD14), circulatory proinflammatory cytokines (including TNF-α, IL-1β and IL-6), and transient disruption of tight junctions in the GI track, which indicated a transient increase in bacterial translocation across the GI tract and systemic activation of the innate immune system (153). HS was also shown to cause a breakdown in the containment of Gram negative bacterial products, as measured by circulating LPS (153). The combined treatment of a 2 Gy dose of either γ-ray or SPE-proton radiation with HS, led to a greater and more sustained elevation in the level of LPS in the serum. Bacterial translocation is known to increase circulating levels of LPS and other bacterial components, which include bacterial DNA (154). To determine whether the increase in LPS induced a systemic response, LBP, which is a type 1 acute phase protein, was measured in ICR mice exposed to radiation with and without hindlimb suspension. LBP is a circulating protein that binds to LPS of Gram-negative bacteria; it is constitutively present but can be induced to higher levels during various types of infection and inflammatory processes. LBP was increased after treatment with proton radiation or HS and was increased further when these stressors were combined (152). Similar results were observed for sCD14, another very sensitive marker of increased levels of circulating LPS. Circulating levels of interferon-alpha (IFN-α) were measured, and at least additive levels of IFN-α were observed for mice treated with both radiation (2 Gy of γ-ray or proton radiation) and HS. These results demonstrate that circulating LPS, resulting from exposure to SPE-like radiation, HS or both, led to a systemic response. It has been concluded from these studies that there is a synergistic effect when hindlimb-suspended mice are additionally exposed to SPE-like radiation.

To determine the mechanisms involved in the increased bacterial translocation across the GI tract, immunohistochemical staining for the tight junction protein, Claudin-3, was performed on terminal ileum sections of mice, and a significant increase in the number of breaks and reductions in staining was observed in mice exposed to proton or γ-ray radiation and HS, as illustrated in Figure 4. These studies indicate that SPE-like radiation and hindlimb suspension induced breaks in the GI epithelial barrier, and suggest that the increased frequency of breaks could be responsible mechanistically for the increase in translocation of bacterial products. To further substantiate this association, the terminal ileum was stained with two antibodies that recognize LPS, a mouse monoclonal antibody against E. Coli (j5) LPS and a goat anti-lipid A IgG that cross-reacts with *Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli 0157, Salmonella enteriditis, Enterobacter aerogenes, E. Hermanii, Yersinia enterocolitica and Shigella sonnei*. Control animals demonstrated no LPS specific staining in the intervillous space, while low levels were observed in mice treated with SPE-proton radiation or HS alone. For the mice exposed to the combined treatment with SPE-proton radiation and HS, there was a high level of diffuse staining, as shown in Figure 5. Thus, the conclusion from these studies was that SPE-like radiation and hindlimb suspension induced the accumulation of LPS in subepithelial regions of the ileum.

It has been estimated that astronauts could receive a dose of up to 2 Gy to the bone marrow from SPE radiation (7, 155). As discussed above, when a 2 Gy dose of radiation is combined with simulated microgravity, an enhanced and prolonged impairment of commensal bacteria containment was observed. We have identified the mechanism for the loss of containment, which is that radiation plus HS leads to breaks in the tight junctions between GI tract epithelial cells, which results in the migration of LPS into the subepithelial tissue. Potential therapies to treat this immune defect could target the GI defect that leads to bacterial translocation or by reducing the inflammatory activity of translocated bacterial products. The mucosal integrity of the GI tract is maintained by a population of $CD4^+$ cells that produce IL-17 (Th17 cells). Their loss is known to be correlated with increased bacterial translocation (156). Although there are no current therapies that can mitigate the loss of GI Th17 cells, this is an area of research worthy of investigation. Antibiotics can be used to treat the increased bacterial translocation, and they are known to be capable of reducing serum LPS levels (157).

Numerous immune system alterations have been associated with space flight in humans and in animals during ground-based spaceflight models (e.g. HS), as has been reviewed [e.g., (10, 158, 159)]. The major effects of spaceflight on the immune system have been well-characterized, and include changes in cytokine production, leukocyte subset distribution and antibody production (10). Examples of cytokines released in response to stimulation include the following: an increase in anti-inflammatory cytokines and a decrease in TNF-a in LPS stimulated spleen cells (160), reductions in interferon-γ and IL-2 following phorbol 12-myristate 13-acetate and ionomycin stimulation of peripheral blood cells of astronauts (21) and reduced NK cell number and function (10, 20). Such alterations in immune function are similar to those brought about when there is increased immune activation produced by exposure to pathogen associated molecular patterns (PAMPs), such as LPS (161–166). Specific examples of this are as follows: a reduction in proinflammatory cytokine production by myeloid cells (166, 167), a reduction in antigen-specific T cell effector cytokine responses (161) and a reduction in circulating NK cells (168). The changes in responses brought about by exposure to PAMPs are known as “tolerance”, in which subsequent responses are reduced in quality and quantity, which is thought to protect the host by limiting excessive inflammation and preventing septic shock (166, 169). As has been observed in many different disease states (154, 162,163, 170–173), continuous or extended exposure to PAMPs can lead to long-lived immune dysfunction. This condition may exist during space flight, as LBP, a well-known marker of immune activation, is known to be elevated in astronauts’ plasma (159, 167). It is possible that the elevated blood levels of LBP resulting from exposure to SPE radiation and simulated microgravity (HS) may bring about immune dysfunctions of the sort that have been observed during and after extended spaceflight.

2.2.2. Effect of SPE-like Radiation on Skin Immune Function—Two models were used to evaluate the effects of SPE radiation on skin immune function. One involved Yucatan minipigs, as their skin histological structure is an accurate model of human skin, and the other involved mice, in which in depth investigations of alterations in immune function could be performed. A major difference between the models used involves the

depth of radiation penetration. The pigs were exposed to electrons or protons as an SPE-like inhomogeneous dose of radiation, with a relatively high dose delivered to the epidermis and dermis and lower doses delivered to internal organs (7), while the mice received proton or reference radiation (γ-rays) exposures as a homogeneous dose of radiation. The pigs were exposed to skin doses as low as 2.5 Gy to up to 25 Gy, which was the highest skin dose evaluated in these studies. It should be pointed out that skin doses for major historical SPEs were calculated to be as high as 32 Gy (7); however, such a high skin dose would represent a worst case scenario that would not be expected to occur frequently. Delayed type hypersensitivity (DTH) responses to phytohemagglutinin (PHA) and LPS were measured prior to radiation and at 7, 14 and 30 days post-irradiation. The responses were symmetric and recorded as the average distance across for induration, erythema and ulceration. Since a similar pattern was observed for all doses of radiation used, with significant increases in the response after radiation, but with no dose dependency, we analyzed all radiation dose groups together for the response to control (PBS), PHA and LPS. A significant enhancement in the DTH response to PHA was observed at all post-irradiation time points evaluated. The responses to LPS were not significantly elevated at day 7, but they become statistically significant at 14 and 30 days post-irradiation (130). The appearance of ulceration after radiation exposure was noted for both PHA and LPS treatments. It is assumed that ulceration occurred as part of the enhanced immune response post-irradiation. If radiation was responsible for the ulceration, ulceration should have increased with increasing doses of radiation, which was not the case. Mice were exposed to a homogenous dose of radiation up to a 2 Gy dose. Mouse skin challenged with intradermal PHA was measured and a similar increase in DTH reactivity was noted after exposure to 2 Gy of proton or γ-ray radiation [(174) and (*Weissman, D., Unpublished data)*].

Skin is known to contain high frequencies of FoxP3 expressing regulatory CD4+ T cells (Tregs). In the skin from irradiated pigs and mice, immunohistochemical analysis demonstrated a loss of $CD3^+$ and $CD25^+$ cells. To determine whether loss of Tregs was responsible for the enhanced DTH responses, RNA was isolated from murine skin prior to and at 2, 7 and 14 days post-irradiation for quantitative PCR measurements of CD3, FoxP3 and GAPDH mRNA. The results demonstrate that there was a statistically significant reduction in FoxP3 mRNA at all 3 time points following exposure to 2 Gy of irradiation. Smaller decreases in $CD3^+$ cells were observed, demonstrating that FoxP3 positive cells were being selectively lost (*Zhou, Y., Ni, H., Balint, K, Sanzari, J.K., Dentchev, E., Diffenderfer, E., Wilson, J., Kennedy, A.R., Cengel, K.A. and Weissman, D., Unpublished data*). Mouse skin was obtained at various time points post-irradiation in the experiment and single cell suspensions of lymphoid cells were obtained by enzymatic digestion for analysis of CD4, CD25 and FoxP3 cells by flow cytometry. Statistically significant decreases in the percent of CD4+ T cells expressing CD25 and FoxP3 were observed at all post-irradiation time points evaluated. The greatest loss was observed at 4 days post-irradiation, with a slow increase in Tregs over the next 28 days. The proliferation of skin $CD4^+$ T cells increased with the loss of Tregs, demonstrating a functional effect.

The loss of skin Tregs could be due to the cell killing effects of radiation or alterations in trafficking. We examined splenic lymphoid cells and observed that, with the loss of skin

Tregs post-irradiation, there was a statistically significant increase in the percent of Tregs in the spleen. The increase in Tregs led to a drop in the proliferation of activated $CD4^+$ and $CD8^+$ cells. A recent report has indicated that Tregs traffic through skin and that they are the main types of cells exiting skin during inflammation (175). In these studies, it was observed that half of the skin cells that migrated to draining lymph nodes were Tregs at steady state. Tomura et al. also noted that when an immune reaction was induced in the skin, the frequency of Tregs draining to lymph nodes increased significantly and made up the majority of cells exiting the skin (175). In addition, it was found that the increase in Tregs leaving the skin resulted in more suppression of T cell activation in the draining lymph nodes and spleen where Tregs accumulated.

To identify mechanisms for the radiation induced depletion of skin T cells, inflammatory genes in mouse skin obtained prior to and at various time points after mice were treated with a single radiation dose of 2 Gy were analyzed. It was observed that at 6 hours post-irradiation, multiple acute inflammatory markers, CXCl chemokines, were upregulated. At 24 hours and continuing through day 14, chronic inflammatory markers, CCL chemokines, complement and IL-10, were induced. These data indicated that irradiation at a dose of 2 Gy induces long-lived inflammatory changes in the skin, including alterations in chemokines known to attract Tregs to the site of infection, including CCL17 and CCL22 (176), which are down-regulated. We hypothesize that the radiation induced inflammation establishes an environment that induces T cells, namely of the regulatory phenotype, to leave the skin and reduce their ability to return to the skin. The lack of Tregs in the skin likely results in a loss of control of the inflammatory response induced by PHA and LPS challenges, resulting in enhanced responses post-irradiation. The clinical significance of this for an astronaut exposed to SPE radiation is unknown. Potentially, as skin abrasions occur during space flight, an enhanced inflammatory response in the setting of reduced immune competence due to Treg migration to lymphoid organs could result in a reduced ability to control an infection.

2.2.3. Effect of SPE Radiation and Hindlimb Suspension on Immune Function Measured by Bacterial Challenge—Infections of the skin, eyes and respiratory tract are common in astronauts: infections have been reported 13 times in the Apollo and 8 times in Skylab missions, and spacecrafts need to be equipped with numerous antibiotics for treatment of such infections (177, 178). A number of bacterial infections have been observed in astronauts during or soon after missions, with organisms that do not typically lead to such infections in healthy people. As one example, *Pseudomonas aeruginosa,* which does not ordinarily infect healthy people, was identified as the pathogen that caused a serious life threatening urinary tract infection in an astronaut during the Apollo 13 mission (179–183). As the control of infections during spaceflight is a major problem, much effort has been focused on determining the effects of spaceflight stressors, such as simulated microgravity and SPE-like radiation, on the ability to defend against a bacterial challenge (151). In these studies, mice were exposed to SPE-like radiation and/or HS, and then challenged with *Pseudomonas aeruginosa* systemically or *Klebsiella pneumoniae* by inhalation. Numbers of bacteria that allow most to all of the untreated animals to survive were used in these studies so that any decrement in immune function could be measured by increased amounts of

bacteria in the blood and lung and morbidity. Three different strains of mice were used: ICR mice are an outbred strain initiated in 1948 from Swiss mice, C3H/HeN mice are inbred and have no known defects of polymorphisms that impair DNA repair or the response to ionizing radiation, whereas Balb/c mice have 2 different polymorphisms in DNA-dependent protein kinases (DNA-PKcs) that mediate non-homologous end joining, which results in decreased, but not absent, function (184–186). To measure the effect of the HS stress and SPE-like radiation on the ability of different strains of mice to effectively clear a challenge with bacteria, hindlimb suspended and/or irradiated mice were exposed 5 days later to *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*. The mice were followed daily for signs of systemic and pulmonary infections. The results for all three strains of mice were comparable and indicated that the mice exposed to HS and SPE radiation failed to control a challenge with *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*, which led to a high morbidity/mortality rate (151), as illustrated in Figure 6 [data from (151)] (Note: in these studies, morbidity was the same as mortality). Either SPE radiation or HS alone had some effects on morbidity in these studies, but when combined, they led to almost complete morbidity/mortality. Other studies in this series of experiments led to the following conclusions: 1) similar levels of morbidity were observed after the challenge with *Pseudomonas* bacteria in male and female mice, indicating a lack of gender/sex differences in this effect, 2) a dose of 1.5 Gy of total body radiation impaired the ability of mice to control the bacterial challenge in a fashion similar to that observed for 2 Gy with little difference in the morbidity observed between these two dose groups, but the morbidity differences observed for the 1 Gy dose group compared to those from the control group were not statistically significant, indicating that the threshold dose for morbidity resulting from the bacterial challenge is between 1 and 1.5 Gy, 3) the relative increases in morbidity were similar for all 3 strains of mice, suggesting that the polymorphisms in DNA-PKcs in Balb/c mice did not significantly affect the response to a bacterial challenge, and 4) peripheral blood granulocyte counts were determined in C3H/HeN mice challenged with *Pseudomonas aeruginosa* at time points prior to HS and irradiation and before and after the bacterial challenge. In these studies, there was a reduction in the peripheral blood granulocyte counts observed post- irradiation, which was similar to that described previously (8, 133, 146). The numbers of peripheral granulocytes increased as expected after bacterial challenge in control or irradiated mice, but the mice treated with HS, with or without radiation, failed to elevate the blood granulocyte counts as expected (151). Similar blunting of the peripheral blood granulocyte counts in response to systemic infection in HS animals was also observed in Balb/c and ICR mice (Drew Weissman and colleagues, unpublished data).

2.2.4. Summary of the Effects of SPE Radiation on the Immune System—The effects of SPE radiation on immune system parameters are of great importance to the space program as they are potentially life-threatening at doses that could conceivably be received by astronauts during space travel. It has been observed that exposure to SPE radiation along with HS, with additional exposure to a bacterial challenge, leads to a very high level of morbidity, which is equal to mortality, in the studies performed in mice. The bacterial challenge utilized bacteria known to be associated with astronaut infections and already part of the spacecraft environment (*Pseudomonas aeruginosa*) or that are part of the normal bacterial flora of the mouth, skin and intestines (*Klebsiella pneumoniae*); the bacterial

challenge utilized in the mouse studies described above was performed with bacterial levels that are non-toxic (or minimally toxic) to the normal control mice. Under the conditions described above leading to morbidity/mortality in the mice, death could be prevented by treatment with an antibiotic (enrofloxacin). Enrofloxacin is approved by the FDA as a veterinary antibiotic (marketed by the Bayer Corporation under the trade name Baytril). A similar antibiotic (ciprofloxacin) is in wide use in human populations. While the effectiveness of the antibiotic is outstanding, the major problem associated with the use of antibiotics for bacterial infections in astronauts is that organisms resistant to the treatment will grow out in a very short period of time [as short as a one-week period of time (Personal Communication, Drew Weissman, M.D., Ph.D.)]. Thus, it is expected that in the exploration class missions of the future, the antibiotics are likely to be less effective with repeated applications over long periods of time. Numerous infections have already been documented in astronauts and are considered a major hazard for spaceflight; some infections have been minor while others have been serious and life-threatening, including a debilitating dental infection and an incapacitating urinary tract infection (158, 159, 183, 187). It is known that spaceflight conditions alter the gene expression patterns, virulence and virulence phenotypes of bacterial pathogens (188), with virulence characteristics increasing with increasing time when cultured under space flight conditions; thus, longer space flights are likely to lead to considerably more serious immunological problems than observed so far in the space program.

The other immunological issue of great importance to the space program is the observation of additive or synergistic synergism in adverse effects caused by SPE radiation exposure and simulated microgravity conditions; interactive effects between SPE radiation and simulated microgravity have been observed for various immunological parameters at doses of radiation that could be received by astronauts during space travel [e.g., (136, 138, 151, 152)]. The results indicate that, under simulated microgravity conditions, the effects of a given dose of SPE radiation can be considerably more severe than the effects observed for the same dose of radiation in normal, control animals. These results suggest the possibility that, in the space microgravity environment, the effects of a given dose of SPE radiation could be comparable to those observed for a significantly higher dose of radiation.

2.3. Emesis

The early phase of the acute radiation syndrome, which is known as the prodromal syndrome, can include nausea, retching, vomiting, diarrhea, and fatigue (1). These effects often manifest within 1 to 72 hours post-irradiation at sub-lethal doses, with a latency time inversely correlated with dose. Vomiting is the reflexive act of forcefully ejecting the stomach contents through the mouth by coordinated muscle contraction. Published clinical studies have demonstrated that patients receiving total body irradiation or upper-abdominal irradiation often show nausea, retching and vomiting as side effects (189, 190). A strong correlation between retching and vomiting events has been established in the ferret model (191, 192). Emetic responses to various pharmacological agents, cytotoxins and radiation have been compared previously among humans and various animal species including nonhuman primates, dogs, cats, and ferrets (191). Ferrets are considered to be a useful species in emesis research (121), especially for radiation and cytotoxic drug-induced emesis

(192), and data from the ferrets have been used by the Department of Defense to develop a mathematical model for the human emetic response to radiation (193). Another advantage of the ferret model is that the prodromal response appears at lower radiation doses and with an earlier onset time as compared to other species, including humans (122). Thus, we have chosen ferrets as an experimental model system to determine the effectiveness of protons at the energy, doses and dose rate ranges relevant to the SPE radiation exposures expected during space travel.

In our studies performed with female descented Fitch ferrets aged 12 to 16 weeks, irradiation with ^{60}Co γ-rays or 155-MeV protons at a high dose rate of 0.5 Gy/minute resulted in dose-dependent changes in the endpoints related to retching and vomiting, such as the fraction of animals that retched or vomited, the number of retching and vomiting events, the length of the latency period leading to the first retching or vomiting event and the duration between the first and last retching or vomiting events (194). A dose-response relationship was observed for ferret vomiting and retching at the high dose rate. The minimum radiation doses required to induce statistically significant changes in the retching- and vomiting-related endpoints were 0.75 and 1.0 Gy, respectively; thus, these values are considered the threshold doses for radiation induced retching and vomiting in the ferret model. The RBE of the proton radiation at the high dose rate did not differ significantly from 1. Similar, but smaller and less consistent, changes in the retching- and vomiting-related endpoints were also observed for ferrets irradiated with γ-rays and protons delivered at the low dose rate of 0.5 Gy/hour. Since this low dose rate is similar to a radiation dose rate expected during an SPE, these results suggest that the risk of SPE radiation-induced vomiting is low and may reach statistical significance only when the radiation dose reaches 1 Gy or higher.

Ferrets have also been used previously to study emesis induced by radiation with ^{60}Co γ-rays (122, 195), 0.6-GeV/n ^{56}Fe ions, neutrons (195) and 200-MeV protons (196, 197), and the emetic response in ferrets was found to be dependent on the type and dose of radiation. High LET ^{56}Fe particles and fission neutrons were comparable in their ability to produce emetic responses (retching or vomiting) in ferrets with an ED_{50} of 0.35 Gy and 0.40 Gy, respectively (195), whereas γ-rays were shown to be intermediately effective with an ED_{50} of 0.77 Gy (122) to 0.95 Gy (195), and high energy electrons were the least effective, with an ED_{50} of 1.38 Gy (195). The ED_{10}, ED_{50} and ED_{90} values estimated for the fraction of animals that vomited after proton irradiation at the HDR were comparable to the ED_{10}, ED_{50} and ED_{90} values after γ-ray irradiation at the high dose rate in our study (194) or at a dose-rate of 1 Gy/minute, as reported previously (122). The ED_{10} and ED_{50}, but not the ED_{90}, values estimated for the fraction of animals that retched (or vomited) post- irradiation with protons at the HDR were lower than the lower limits of the respective 95% confidence intervals previously reported for γ-rays (122), suggesting that HDR proton irradiation was more effective than HDR γ-ray irradiation in inducing retching and vomiting.

2.4. Effects on Radiation on Blood Coagulation and the Development of Disseminated Intravascular Coagulation

Relatively little information exists in the literature on the effects of radiation on blood coagulation. Blood coagulation involves multiple components, which generate a fibrin-rich blood clot to stop bleeding in a process known as hemostasis. Primary hemostasis starts with the activation of platelets at the wound site by exposing collagen to blood, which allows von Willenbrand factor (vWF) to bind to collagen and tethers platelets to the vasculature wall. The coagulation pathway, which is also referred to as secondary hemostasis, occurs simultaneously on the negatively charged surface of activated platelets to generate a fibrin-rich thrombus (198). The exposure of tissue factor (TF) at the injury site of blood vessels, and its subsequent binding with Factor VII (extrinsic pathway), initiates a thrombin (Factor II) burst that leads to fibrin clot formation through the activation of a series of vitamin K-dependent serine proteases. It is the activation of coagulation factors, such as Factor V and VIII, by thrombin that drives the development of a stable fibrin clot as a part of the intrinsic pathway (199, 200).

Previous studies have demonstrated that radiation can induce vWF secretion from human umbilical vein endothelial cells irradiated in tissue culture (201) and the vWF mRNA levels were increased when either human or bovine endothelial cells were exposed to 20 Gy irradiation with a 6-MeV electron beam at a fixed dose of 2.4 Gy/minute (202). It has also been shown that in human peripheral blood mononuclear cells (PBMNCs) after irradiation with a 6-MeV electron beam, TF was up-regulated, which led to a significant increase in PBMNC-associated procoagulant activity over a time period of 7 days post-irradiation. Increased cellular TF protein concentration was observed up to 7 days post- irradiation, and microparticle-associated TF activity was increased significantly 3 days post-irradiation as compared with the non-irradiated controls. PBMNC-derived microparticles post-irradiation also initiated the plasma clotting faster than microparticles derived from controls. The radiation induced TF expression and increase in procoagulability of PBMNCs and cell-derived microparticles may represent a possible mechanism by which ionizing radiation enhances blood thrombogenicity (203). In a study performed with leukoreduced fresh-frozen plasma irradiated with 30 Gy of γ-rays, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time, antithrombin III, protein C, protein S, vWF, ristocetin cofactor, plasminogen–α2-antiplasmin, the coagulation factors fibrinogen, FII, FV, FVII, VIII, F IX, FX, FXI, FXII, FXIII, and activated factor XII (FXIIa), D-dimer, fibrin monomer, thrombin–antithrombin complex, prothrombin fragment 1 + 2 (F1+2), plasmin–α2-antiplasmin complexes, and platelet factor 4 were determined, and PT, aPTT and FVIII activities were found to be decreased significantly, whereas activities of the coagulation factors FII, FV, FVII, FIX, FX, FXII were increased significantly post-irradiation (204).

To determine whether SPE-like proton radiation could affect blood clotting times, we have performed experiments with 12 to 16 week old descented female Fitch ferrets and demonstrated significant increases in the PT at 3 hours post- irradiation with doses of 1 or 2 Gy (but not 0.25 Gy) of 110-MeV protons delivered at a high dose rate of 0.5 Gy/minute or 0.25 and 1 Gy (but not 2 Gy) of 110-MeV protons delivered at a low dose rate of 0.5 Gy/

hour (205). Human PT values are commonly reported as an INR which is defined as the patient's 'test' PT value divided by the laboratory 'normal' PT value, raised to the power of the International Sensitivity Index. INR values were calculated for animals exposed to 2 Gy of 110-MeV protons at both the high and low dose rates and 1 Gy of 110-MeV protons at the low dose rate, which resulted in the greatest PT response to proton radiation in the study. Three out of 10 animals exposed to 1 Gy at the low dose rate had an INR value of ≥ 2.0 and an additional 3/10 of the ferrets had borderline INR values (> 1.75) approaching 2.0 (205), which is considered to be of clinical concern for humans (206, 207). The INR values for the animals exposed to 2 Gy protons at the high dose rate were significantly higher than the pre-irradiation levels, although they were still below 2. In addition to the increase in PT, aPPT also increased 3 and 48 hours after 0.25, 1 or 2 Gy of 110-MeV proton irradiation at the low dose rate (205). For the ferrets irradiated with 110-MeV protons at the high dose rate, significant increases in aPPT was not observed in any of the radiation dose groups at 3 hours post-irradiation or in the 0.25 and 1 Gy dose groups at 48 hours post- irradiation. The increase in PT induced by the proton irradiation at the high dose rate was due to Factor VII whereas Factors II, V, VII IX contributed to the increases in PT induced by proton irradiation at the low dose rate (205). These results demonstrated that proton irradiation significantly increased blood clotting times due to different coagulation factors, indicating potential radiation-induced coagulopathy. The finding that the effects of the proton radiation at the low dose-rate are more severe than those at the high dose-rate on an endpoint is an unexpected finding in radiobiology, as the expectation is that reducing the dose rate will have a sparing effect, thereby reducing the severity of effect on the biological endpoint being evaluated. Thus, the increased effect of low dose-rate irradiation on ferret blood clotting times is particularly noteworthy.

The blood clotting abnormalities in the ferrets are thought to lead to a condition known as disseminated intravascular coagulation (DIC), which is believed to result in 100% mortality in ferrets irradiated with a 2 Gy dose of either γ-ray or SPE proton radiation (142). The increases in blood clotting times became more evident in ferrets destined to die from exposure to radiation. The LD_{50} dose for the development of DIC in ferrets is 1.5 Gy (142).

While irradiation with SPE-like protons was shown to increase prothrombin time and partial thromboplastin time (205), the mechanism for the proton radiation induced hypocoagulation remains to be elucidated. We have hypothesized that the SPE-like proton irradiation activates the coagulation cascade, which would put irradiated subjects in a hypocoagulable state. To test this hypothesis, a separate experiment was performed with 12 to 15 week old de-scented female ferrets irradiated with 1 Gy of 110-MeV protons at a dose rate of 0.5 Gy/hour, and the results indicate that the radiation exposure resulted in coagulation cascade activation, which was indicated by increases in soluble fibrin concentration in the blood and fibrin clots in blood vessels of livers, lungs and kidneys from irradiated ferrets (208). The soluble fibrin concentration was determined using a rapid soluble fibrin assay that was previously developed and implemented at the Loma Linda University Medical Center to aid in early detection of DIC in emergency room, operating room, or transplant patients (209). In addition to the activated coagulation cascade, PT and aPTT were also increased after irradiation, which is indicative of the involvement of the extrinsic/intrinsic coagulation pathways. The platelet counts in the irradiated ferrets remained at approximately pre-

irradiation values for up to 7 days post-irradiation, indicating that the observed effects on blood clotting times were not platelet-related. The activation of the coagulation cascade is expected to consume clotting factors, which, in turn, leaves the animal deficient in clotting factors. Thus, the increased PT and aPTT values in the irradiated animals might have been due to radiation-induced effects on secondary hemostasis. WBC counts were reduced significantly within 24 hours post- irradiation and they remained reduced up to 7 days post-irradiation with a dose of 1 Gy of SPE-like proton radiation.

DIC is a serious, life-threatening condition in which clotting and bleeding are occurring at the same time, and it is often fatal due to multiple organ failure. Mechanistically, activation of the clotting cascade is expected to decrease the bioavailability of the factors in the blood, thereby increasing the PT/aPTT values, as was observed in the irradiated ferrets (205, 208). Radiation exposure may significantly decrease leukocyte counts, and the prolonged low WBC, neutrophil, and lymphocyte counts can leave the irradiated subjects at risk for infection, thereby further overwhelming hemostasis and potentially leading to DIC, as has been observed in patients with sepsis. Currently, the mechanism of radiation-induced death at the dose expected to kill 50% of the irradiated subjects (LD_{50}) is thought to be due to bone marrow cytotoxicity (known as the hematopoietic syndrome), which results in a dramatic reduction in the number of circulating hematopoietic cells and the resultant symptoms of infection (from white blood cell loss) and bleeding (presumably from platelet loss) (210). However, studies performed with ferrets have suggested that the death of the animals irradiated at the LD_{50} dose is due to a consumptive coagulopathy, which is followed by the onset of DIC, since hypocoagulation occurred during early time points post-irradiation when the platelet counts were at normal levels (142). The ferret study results have shown that exposure to proton or γ-ray radiation produced step-wise changes in hemostasis that begin with the radiation activated clotting cascade, which results in the cleavage of fibrinogen and the formation of fibrin clots. Activation of the coagulation cascade also leads to increased PT and aPTT values due to the consumption of associated factors (205). These changes occurred as early as 3 hours post-irradiation, along with the detectable soluble fibrin in the blood as well as detectable fibrin clots in the blood vessels of irradiated tissues (208). In animals destined to die after irradiation, these abnormal hemostasis parameters became progressively worse. In ferrets exposed to a lower (sub-lethal) radiation dose (e.g., 1 Gy), the abnormal hemostasis characteristics recovered steadily and by day-30 post-irradiation, the clot formation, clot size, and platelet clumping values returned to baseline. These results are particularly important, since the recognition of radiation induced DIC as a cause of death could change the course of actions when the acute radiation syndrome is diagnosed and treated in people exposed to radiation through occupational accidents, radiation terrorism or other catastrophic events (142).

It is noteworthy that experiments performed as part of this project also documented the development of DIC in Yucatan minipigs (*Krigsfeld, G.S., Shah, J.B., Sanzari, J.K., Lin, L and Kennedy, A.R., Unpublished data*). Three pigs were irradiated with a 2.5 Gy homogeneous dose of total body x-irradiation. One of these pigs died, and another was euthanized; both of these pigs were diagnosed with DIC. The third pig did not die, but exhibited severe blood clotting abnormalities (greatly increased bleeding times, etc.), as did

the two pigs that did not survive. In addition, three pigs were irradiated with a 2 Gy total body dose of SPE-like proton radiation. These pigs exhibited severe blood clotting abnormalities (increased blood clotting times), but they survived the radiation exposure.

2.5. Effects on Radiation on Fatigue

Several experiments have been performed to evaluate the ability of SPE proton and γ-ray radiation to induce fatigue in mice, as reflected in changes in social exploration, submaximal exercise treadmill and locomotor activity (211–213). The results of studies on social exploration indicated that low, but not high, dose-rate γ-ray and proton radiation exposures led to comparable transient increases in social withdrawal, and these effects are thought to be due to a combination of restraint stress and radiation. The results for studies on submaximal exercise treadmill indicated that neither γ-rays nor protons impaired performance on this test. In a study performed with 7 to 8-week old male CD-1 mice irradiated with 0.5 or 2 Gy ^{137}Cs γ-rays at a dose rate of 44.5 cGy/minute (high dose rate), ^{60}Co γ-rays at a dose rate of 0.5 cGy/minute (low dose rate) or protons at dose rates of 0.5 Gy/minute (high dose rate) or 0.5 cGy/minute (low dose rate), locomotor activity was reduced in mice irradiated with γ-rays at the high dose rate but not in the mice irradiated with γ-rays at the low dose rate or with protons, which had a similar macroscopic dose distribution as that from ^{60}Co γ-rays exposure, when delivered at either the high or the low dose rate (211). The γ-ray irradiation also increased hippocampal TNF-α expression, which occurred as early as 4 hours post-irradiation and was followed by subsequent increases in IL-1RA in the cortex and hippocampus and reductions in activity-regulated cytoskeleton-associated protein (Arc) in the cortex. These observations indicate that low-dose ionizing radiation rapidly activates the neuroimmune system, potentially causing early onset fatigue-like symptoms in the irradiated animals.

In these studies related to proton or photon induced fatigue, the results were consistent with a threshold effect, i.e., once a dose sufficient to produce a response is given, additional dose does not increase the magnitude of the response. The magnitude of the response with γ-rays is small, and additional stressors, such as restraint or other manual manipulations, are sufficient to obscure any acute behavioral changes. For those tests in which altered behavior was noted for γ-rays, protons showed a similar trend, which did not reach statistical significance. Therefore, the RBE for proton radiation on this endpoint is assumed to be less than 1, although it could not be defined mathematically due to the lack of a statistically significant trend in the proton irradiated animals.

The data gathered on the ability of SPE radiation to induce fatigue in mice suggest that exposure to low-dose rate ionizing radiation leads to a minimal increase in fatigue, in the form of depressive/anxious behaviors, and that these effects are transient with full recovery within the 24 hour period (211, 213). These behaviors are likely of equal or lesser magnitude than the depressive/anxious behaviors that are stimulated by the restraint stress necessary to perform the experiments and that is likely to be experienced by astronauts in typical space exploration vehicles. Thus, acutely, SPE radiation up to a 2 Gy total body dose is highly unlikely to increase fatigue or other adverse behaviors over and above baseline levels for astronauts and is therefore highly unlikely to lead to mission critical fatigue.

2.6. Heart Functional Changes

As part of the experiments designed to determine whether a high skin dose from SPE radiation has adverse effects on the internal organs of pigs, we evaluated changes in the heart brought about by a high skin dose of electron radiation planned to simulate a dose distribution pattern like that expected from SPE radiation. As changes in the left anterior descending (LAD, interventricular) artery can have major effects on heart function, our studies in this area of research focused on LAD function in the pig hearts. The LAD artery provides the blood supply to the mid-region of the heart and is a major site of vessel stenosis. Vessels from control and pigs exposed to electron radiation exhibited a similar relaxation response following treatment with adenosine diphosphate and sodium nitroprusside. There was a reduced relaxation response to bradykinin (BK) treatment in the arteries from hearts exposed to SPE-like electron radiation. In contrast, vessels obtained from control animals exhibited a 20% higher relaxation response with BK, compared with arteries obtained from the irradiated pigs. Denuded vasculature, isolated from untreated and irradiated animals, was unresponsive to BK treatment, confirming that the BK response is mediated via receptors present on the surface of endothelial cells (214). The fact that the relaxation response was lower in irradiated tissue suggests that radiation exposure damages the vascular endothelium. The results of this study demonstrate that radiation has a direct effect on the cardiac vasculature. Intact arteries from hearts taken from irradiated pigs exhibited a reduced contractile force following exposure to BK. BK is an endothelium-dependent dilator that acts directly on endothelial cells, causing them to release nitric oxide, PG12 and perhaps endothelium derived dilating factor(s), which signal relaxation of vascular smooth muscle (215). Our results suggest that alterations in vascular function are primarily a consequence of radiation-induced damage to endothelial cells, and that astronauts exposed to a high dose of SPE radiation could be at risk for vascular damage in the heart. While the skin dose was high in this study, the heart dose was only 0.35 Gy. This heart dose is considerably lower than the doses of radiation (0.5–5 G) previously shown to affect endothelial dependent relaxation in rat aorta (216, 217).

2.7. Skin Effects

The skin effects in pigs have been described previously (130) and the immunological changes in pig skin accompanying the skin changes are described above in the section on immunology effects. It has been observed that pigmentation increases with increasing exposure to SPE radiation (130), and a method of quantitation for the melanin changes in irradiated pig skin has been developed (*Billings, P.C., Sanzari, J.K., Kennedy, A.R., Cengel, A.K. and Seykora, J.T., Unpublished data*). With very high skin SPE radiation doses, there is evidence of blood vessel loss in the pig dermis, as illustrated in Figure 7. A consequence of this radiation effect is a reduced blood flow to these areas of the dermis. It is known that areas of the dermis receiving a reduced blood flow can develop into a “diabetic foot” like state (which needs surgical removal) in patients undergoing interventional radiology procedures (218, 219). In humans, the development of such lesions requiring surgical removal takes approximately one year or more and the skin doses associated with such lesions are high (≥ 10 Gy) (219). Such lesions could conceivably be a problem during the multi-year exploration class missions planned for the future.

2.8. Increased Intracranial Pressure and Potential Vision Abnormalities Evaluated in Yucatan Mini-pigs

Vision changes, characterized as a degradation in distant and near visual acuity, have been documented in numerous astronauts who have been involved in long-duration (of six months or longer) space flight (220). Although the etiology of the vision alterations is unknown, Mader et al. hypothesized that the optic nerve and ocular changes observed in astronauts could have been caused by prolonged microgravity exposure (220). Since radiation exposure in clinical radiotherapy patients is known to be associated with increased intracranial pressure, increased radiation exposures during space travel could also contribute to the alterations thought to be involved in producing the vision abnormalities. We have evaluated the ability of SPE-like radiation in pigs to produce changes like those associated with the astronaut vision alterations, by performing ocular ultrasound examinations, lumbar puncture opening pressure studies and histopathologic examinations of eye tissue taken from pigs irradiated with SPE-like radiation (in the forms of simulated electron (6 + 12 MeV) SPE (eSPE) or simulated proton SPE (pSPE) exposures (144)). The dosimetry studies have indicated that the eyes and lenses receive substantial doses of radiation during an SPE, which are roughly comparable to the doses received by the skin of pigs (e.g., see Fig. 1).

2.8.1. Histopathology Changes in the Eyes of Pigs Exposed to Simulated Proton and Electron SPE Radiation Which Could Be Related to Vision Problems—

The retina is a layered structure of neurons interconnected by synapses sending information to the brain via the optic nerve. The retina includes millions of photoreceptor cells, known as rods or cones, which are sensitive to light. Change or damage to the retina can cause loss of vision. Signs of damage to the retina are sudden flashes of light, floating spots, decreased vision, or distorted vision. The ocular histopathology of pigs exposed to pSPE (5–10 Gy) or eSPE (5–20 Gy) radiation was examined. In this study, (Sanzari, J.K., Zeiss C.J., and Kennedy, A.R., Unpublished data) sagittal sections of the entire eye and cross sections of the optic nerve were prepared and qualitative differences (as well as some quantitative measurements) between the eyes of the irradiated and non-irradiated animals were investigated.

It was determined that eSPE radiation exposure resulted in a decreased total retinal width in 20% of the irradiated animals compared to the non-irradiated control animals; the differences observed in these studies between the irradiated and control pigs were statistically significant. Changes in retinal width can cause turbulence in blood flow and may indicate atrophy affecting the nerve fiber layer, ganglion cell layer, outer plexiform layer, and inner nuclear layer of the retina. It was confirmed that the width of the outer plexiform layer was also reduced in some of the animals exposed to eSPE radiation. A reduction in the width of the outer nuclear layer is an indicator of photoreceptor cell loss, which was confirmed by the extrusion of photoreceptor nuclei in the retina. The extrusion of photoreceptor nuclei (an indication of active cell death) into the inner segments was observed in both the eSPE and pSPE animals. The dose-response relationship was evaluated for the extrusion of photoreceptor nuclei induced by pSPE radiation exposure and results indicated a statistically significant slope, establishing that the loss of photoreceptor nuclei in pSPE irradiated pigs was dose-dependent.

Upon evaluation of the optic nerve area, the meningeal sheath area was dilated in 31% of the irradiated animals, and the differences between the measured area of dilation in the irradiated pigs compared to the non-irradiated pigs were statistically significant. Further, an accumulation of lymphocytes, plasma cells, and macrophages around the vessels near the optic nerve was observed, indicating an inflammatory response to radiation exposure; however, the presence of inflammatory infiltrates was not consistent in the irradiated animals (inflammatory infiltrates were present in 10% of the irradiated pigs).

The results described above suggest that SPE radiation may result in radiation-induced retinal atrophy or degeneration; however, long term studies will be necessary to determine whether the loss of photoreceptor cells and changes in retinal width or optic nerve area persist at later time periods.

2.8.2. Ocular Ultrasound Results of the Eyes of the Pigs Exposed to SPE Radiation—Noninvasive measurements such as bedside ultrasound have been advocated and utilized in the clinical setting and during spaceflight as a surrogate endpoint biomarker of increased intracranial pressure. Live ocular ultrasound imaging was performed in the pSPE animals approximately 2 months post-irradiation as part of a collaborative study between the investigators at the University of Pennsylvania (Penn) and Wyle/NASA investigators skilled in ocular ultrasound examination techniques. The sonographer at Penn was remotely-guided by an expert in eye/optic nerve imaging, and the remotely guided sessions were conducted in a way that was similar to the “telemedicine” and “telescience“ arrangement routinely used during ultrasound imaging sessions on the International Space Station (ISS). The optic nerve sheath diameter was measured using electronic calipurs at three distances from the vitreoretinal interface. Animals exposed to 5–10 Gy pSPE radiation exhibited a dose-dependent trend in increased diameter measurements compared to the non-irradiated animals. The measurements recorded for the animals exposed to 5 Gy pSPE radiation resulted in a significant increase ($p = 0.02$ for right eye; $p = 0.05$ for left eye), compared to the non-irradiated group of animals. The 10 Gy dose pSPE radiation resulted in significantly increased sheath diameters as well ($p = 0.002$ for right eye; $p = 0.03$ for left eye) (Sanzari, J.K., Sargysan, A.E., Ebert, D., Garcia, K.M., Shultz, S.M., Seghal, C.M., and Kennedy, A.R., Unpublished data). Ocular ultrasound examinations were not performed on the corresponding animals exposed to eSPE radiation (for which histological changes are reported above).

When considered together, the histopathology and the ocular ultrasound exam results indicate that SPE radiation exposure directly affects the eye structure of animals exposed to 5–20 Gy electron simulated SPE radiation or 5–10 Gy proton simulated SPE radiation. The observed changes in the optic nerve area may be associated with the optic disc edema observed during/post spaceflight reported by Mader et al. (220).

2.8.2. Opening Pressure in Pigs Exposed to Proton and Electron SPE Radiation—The opening pressure of pigs exposed to either pSPE or eSPE was measured by lumbar puncture procedures and were found to be increased in some of the irradiated animals, with relatively larger increases in animals exposed to larger electron or proton SPE radiation doses (221). Pigs exposed to lower skin doses of 2.5 to 7.5 Gy eSPE radiation (like

the SPE radiation occurring in October 1972) exhibited increased opening pressure values, which lasted up to 90 days post-irradiation (i.e., at the time when the experiment was terminated), suggesting that SPE-like electron radiation resulted in increased intracranial pressure after a radiation exposure with a skin dose as low as 2.5 Gy (221).

The results in this area of research indicate that exposure to even relatively low skin doses of SPE radiation can result in some of the alterations thought to play a role in astronaut vision alterations (e.g., increased intracranial pressure and increases in nerve sheath diameter). Thus far, astronauts have not been exposed to significant doses of SPE radiation during spaceflight, but it is expected that there will be an increased risk of astronaut exposure to higher doses of SPE radiation in the exploration class missions of the future. It is hypothesized that exposure to SPE radiation along with extended space travel could exacerbate the development of visual changes in astronauts. However, larger numbers of exposed pigs than those used in the studies described above will be necessary to confirm and verify this hypothesis.

2.9. Short-term Survival in Irradiated Animals

In experiments performed with male ICR mice aged 4 to 5 weeks exposed to 5.9, 6.8 or 7.2 Gy of total body irradiation with 1-GeV protons at dose rates ranging from 0.2 to 0.7 Gy/minute, the 30-day survival was 60%, 13.3% and 0%, respectively, and the calculated lethal dose to kill 50% of the irradiated animals was 6.23 Gy (139). In a separate experiment performed with male ICR mice aged 4 to 5 weeks irradiated with 6 or 8 Gy of 225 kVp X-rays, the 30-day survival was 100% and 6.7%, respectively (132); these survival levels are higher than those observed in the 30-day survival studies in the animals exposed to 5.9–7.2 Gy of 1-GeV/n protons. These results indicate that 1-GeV proton radiation is more lethal to mice than X-rays. Similar studies were performed by other investigators in C57BL/6J mice to determine the relative toxicity of HZE radiation (1-GeV/n ^{56}Fe ions) compared to γ-rays or 1-GeV protons (222). In these studies, the $LD_{50/30}$ values for ^{56}Fe ions, protons and γ-rays were reported to be 5.8, 6.8 and 7.25, respectively; the RBE value for the ^{56}Fe ions was 1.25 and for the protons, the RBE was 1.06. It was concluded from these studies that ^{56}Fe ions caused accelerated and more severe hematopoietic toxicity. Of interest in this study was the finding that intestinal crypt cells did not show increased HZE toxicity. In another reported study performed in C57BL/6J mice, it was observed that the $LD_{50/30}$ values for ^{28}Si and ^{12}C ions were 5.17 and 7.34 Gy, respectively (223). In these studies, the RBE values for ^{28}Si and ^{12}C ions (compared to the γ-ray data in which the LD50/30 was 7.25) were 1.4 and 0.99, respectively.

In studies performed with ferrets, the observed 30-day survival was 100% for ferrets irradiated with up to a 1 Gy dose of SPE-like protons and zero for ferrets exposed to 2 Gy of SPE-like protons (142). The $LD_{50/30}$ for SPE-like proton radiation in ferrets was estimated to be approximately 1.5 Gy (142). The survival curves for γ-ray irradiated ferrets were comparable to those for the proton-irradiated animals, and both the proton and γ-ray irradiated ferrets displayed signs of distress including ecchymosis, petechiae, and hemorrhaging. It was hypothesized that the ferrets were dying of DIC in these studies (224), which was confirmed by additional evidence collected (142).

3. Long-term Effects

3.1. Long-term Survival in Irradiated Animals

In a long-term study in which male CBA/JCR HSD mice aged 8 to 9 weeks were irradiated with 3 Gy of 1-GeV protons or 0.5 Gy of 1-GeV/n ^{56}Fe ions and monitored for 2 years after irradiation, the survival of the mice was significantly lower in mice exposed to 3 Gy protons ($p < 0.001$) or 0.5 Gy ^{56}Fe ions ($p < 0.05$) than in mice receiving only the sham radiation (73). The decreased survival in the irradiated mice was accompanied by a significant increase in the rate of development of malignant lymphoma and Harderian gland tumors as well as the fractions of animals with malignant lymphoma or rare tumors (73, 225).

3.2. Cataract Development

In the long-term experiment with CBA/JCR HSD mice exposed to 1-GeV protons at a dose of 3 Gy or 1-GeV/n ^{56}Fe ion radiation at a dose of 0.5 Gy, the mice were observed daily over approximately two years after the radiation exposure. The animals were then euthanized and lenses were harvested and characterized using an established classification system that assigns discrete scores based on the severity of the lens opacifications. The results showed that exposure to 1-GeV/n proton (3 Gy) or ^{56}Fe ion (0.5 Gy) radiation significantly increased the cataract prevalence and severity in CBA/JCR HSD mice to levels above the baseline levels of age-induced cataract formation in this mouse strain (226).

3.3. Cancer Development

Malignancy is considered to be a particular risk associated with exposure to the types of ionizing radiation encountered during space flight. In these studies, the ability of protons and highly energetic, heavy particles (HZE particles) to induce carcinogenesis was determined in CBA mice. The major finding of the studies was that there was an increased risk of developing malignant lymphoma and rare tumor types, including Harderian gland tumors, in animals exposed to space radiations (73, 225). A significant increase in pre-malignant and malignant lesions of myeloid origin was also observed in mice exposed to 3 Gy proton radiation and 0.5 Gy ^{56}Fe ion radiation. These results indicate that exposure to space radiations can increase the incidence of malignant tumors as well as pre-malignant lesions in mice. These studies have been reviewed recently (28).

4. Radiation Induced Changes in Gene Expression

In a study performed with 5–6 week old ICR mice, irradiation with 1-GeV protons was shown to increase the mRNA levels for Bax, caspase-9, caspase-8, NFκB1 and TGFβ1 and protein levels for Bcl2 and Bcl-xL (227). The proton irradiation was also shown to induce cleavage of pro-apoptosis proteins, such as caspase-3 and PARP-1, in bone marrow lysates of the irradiated animals. These results confirm the findings that the high-energy proton radiation can induce the gene expression of classical markers of apoptosis, as well as the downstream effectors, caspase-3 and PARP-1. In a separate study performed with ICR mice irradiated with 1.0 and 6.4 Gy of 1-GeV protons or 1.1 and 7.0 Gy of γ-rays to compare the acute effects of radiation on gene expression in radiation-sensitive tissues (e.g., spleen, thymus, bone marrow, testis and the GI tract), the apoptotic responses were found to vary

greatly between γ-ray and proton irradiated animals in a tissue- and dose-dependent manner and cell death in the splenic white pulp was consistently lower in the proton-irradiated animals compared to the γ-ray irradiated animals (228). Both proton and γ-ray irradiation triggered nuclear accumulation of p53, with no significant differences in the majority of the known pro-apoptotic p53-target genes in the spleens of irradiated mice. However, γ-ray irradiation uniquely triggered a pro-apoptotic expression profile in the spleen and Peyer's patches, which exhibited a higher level of apoptosis after γ-ray irradiation than after the proton irradiation despite the increased presence of DNA strand breaks and phosphorylated-ATM in the spleens of proton irradiated animals. Differences in the acute pro-apoptotic response to proton and γ-ray irradiation correlated with increased expression of the p53-dependent pro-apoptotic gene, Bcl-G, and granzyme B, suggesting that the fate of the cells after proton and γ-ray irradiation may be context-dependent and the triggering of apoptosis in lymphoid cells after irradiation may not be dependent solely on the extent of DNA damage brought about by the radiation exposure.

In female ICR mice irradiated with ^{60}Co γ-rays at low (0.5 Gy/hour) or high (0.5 Gy/minute) dose rates, changes in the expression of genes implicated in oxidative stress, extracellular matrix (ECM) remodeling and selected protein expression profiles in mouse skin were examined using skin tissues harvested at 4 hours post-irradiation (174). After irradiation at doses as low as 0.25 Gy, the expression of many genes responsible for regulating the production of reactive oxygen species were significantly altered by more than 2-fold as compared to unirradiated controls. The expression profiles of 18 to 20 of the 84 ECM genes were also significantly altered after irradiation at the low dose rate. As compared to the low dose rate irradiation, the high dose rate irradiation resulted in different ECM gene expression profiles, with the most striking differences observed for genes encoding matrix metalloproteinases. These results indicate that the expression of many genes involved in oxidative stress responses and ECM remodeling may be differentially regulated by high and low dose rate irradiation.

5. Countermeasures and Mitigation of Space Radiation Damage

It has been well established that the biological effectiveness of ionizing radiation depends on the LET, which describes the rate of energy loss along the trajectory of ionizing particles (229) and the ion species (230, 231). Ionizing radiation damages cells through a combination of direct action, which refers to the direct hit of biologically important targets by the particle radiations, and indirect actions via water-derived free radicals produced by the radiation (232). While the indirect action plays an important role in the biological effects of low-LET radiations, such as X-rays and γ-rays, its contribution diminishes with increase in LET and the direct action contributes more to the biological effects of high-LET radiation than the indirect action (232).

From our studies, there have been many publications related to countermeasures for acute radiation effects (28, 63,73,130, 132, 135, 139, 208, 225–227, 233–235). These publications are briefly described below.

5.1. Radiation Induced Oxidative Stress and Antioxidants as Countermeasures

Using a dichlorofluorescein (DCF) fluorometric assay that we had previously adapted and standardized to measure radiation induced oxidation in live cells (236, 237), we have demonstrated that low LET photon radiation, such as X-rays and γ-rays, and high LET radiation, such as 0.6-GeV/n silicon ions and 1- or 5-GeV/n ^{56}Fe ions, as well as 250 MeV protons, are all capable of inducing oxidative stress (238), suggesting that the indirect actions via free radical generation may contribute substantially to the biological effects of both low and high LET radiation. Since the removal of radiation generated free radicals will make them unavailable to damage DNA, proteins and cell membrane components, antioxidants could be effective countermeasures against radiation induced oxidative stress and other adverse biological effects occurring downstream to the free radical generation.

The agents evaluated in our studies as potential countermeasures for radiation induced oxidative stress included N-acetyl cysteine (NAC), ascorbic acid (or vitamin C), coenzyme Q10, folic acid, glutathione, α-lipoic acid, niacin, L-selenomethionine (SeM), thiamin and vitamin E succinate. NAC is a small molecular weight thiol and a precursor to intracellular cysteine and glutathione (239), which is a tripeptide small molecular weight thiol shown to be a versatile protector against radiation induced oxidative damage (240). NAC is also effective in activating NF-κB and manganese superoxide dismutase (MnSOD) gene expression (241–243); MnSOD is a main mitochondrial antioxidant enzyme with radioprotective properties (244–246). Vitamin C is a water-soluble antioxidant that reacts with highly damaging hydroxyl radicals to form less toxic ascorbate free radicals, which can be detoxified by enzymes that reduce ascorbate free radicals back to ascorbic acid (247). Vitamin E is a lipophilic agent that protects cell membranes from oxidative damage by radiation or other physical or chemical agents (248). Dietary supplementation of vitamins C and E is thought to be important for protection against human diseases associated with free radical damage to cellular DNA, lipids and proteins (249). Lipoic acid is a B vitamin that is both lipid and water-soluble and is considered as a “universal antioxidant” because it can react with hydroxy radicals, singlet oxygen, and peroxyl and hypochlorous radicals (250). It is an excellent radical scavenger both in the oxidized and reduced form and is known to regenerate other antioxidants from their inactive forms. As examples, lipoic acid plays an essential role in mitochondrial dehydrogenase reactions (251). It also protects cell membranes by reacting with and regenerating vitamin C and glutathione, which in turn recycle vitamin E (250). Treatment with lipoic acid has been shown to reduce radiation-induced oxidative stress (250, 252, 253) and hematopoietic tissue damage in irradiated mice (254). Treatment with lipoic acid in combination with vitamins C and E has been shown to protect against lens damage caused by low dose irradiation (255). Selenium is an essential trace element for maintaining activities of the important antioxidant enzymes, thioredoxin reductase and glutathione peroxidase (256, 257). Together with vitamin E, it protects cell and organelle membranes from oxidative damage, facilitates the union between oxygen and hydrogen at the end of the metabolic chain and the transfer of ions across cell membranes (258). Another agent evaluated as a potential countermeasure for radiation induced oxidative stress is a soybean-derived protease inhibitor known as the Bowman-Birk inhibitor (BBI), which has been developed in the form of BBI Concentrate (BBIC) for cancer prevention and human trials, as reviewed previously (259–262). Both BBI and BBIC have been shown to

have antioxidant properties (260) and BBI has been utilized as a radioprotective agent (263–271).

Using the DCF fluorometric assay method previously adapted for measurement of radiation induced oxidative stress in cultured cells (236, 237), we performed experiments with X-rays, γ-rays, protons and HZE particles to evaluate the protective effects of antioxidants against radiation induced oxidative stress and found NAC, ascorbic acid, α-lipoic acid and SeM to be highly effective in preventing radiation induced oxidative stress, whereas coenzyme Q10 and vitamin E succinate were only weakly effective in preventing radiation induced oxidative stress in cultured cells (272, 273). Based on the results of the DCF fluorometric assay experiments, these antioxidants were selected as a combination for further studies *in vitro* and *in vivo*.

The ability of the antioxidant combination and BBIC to prevent radiation induced oxidative stress *in vivo* was evaluated in Sprague-Dawley rats and CBA mice irradiated with γ-ray, proton or ^{56}Fe ion radiation using the total antioxidant status (TAS) in serum or plasma as the biological endpoint (272, 274–276). In the rat studies, the serum or plasma level of total antioxidants was found to be decreased after exposure to γ-ray or 1-GeV/n ^{56}Fe ion radiation, and the decrease was alleviated or completely prevented in the animals fed with a diet supplemented with SeM (12 μg/g diet) alone or in combination with sodium ascorbate (19 μg/g diet), NAC (51 μg/g diet), α-lipoic acid, reduced form (100 μg/g diet), vitamin E succinate (8.6 μg/g diet) and coenzyme Q10 (51 μg/g diet) (272, 274). In the mouse studies, the plasma TAS also decreased significantly after exposure to 0.5 Gy of 1-GeV/n ^{56}Fe ion radiation or 3 Gy of 1-GeV proton or γ-ray radiation, and the decrease in plasma TAS was alleviated or prevented completely by diet supplementation with BBIC (10 mg/g diet), SeM (0.14 μg/g diet), or a combination of L-SeM (0.14 μg/g diet), sodium ascorbate (17.14 μg/g diet), NAC (51.43 μg/g diet), α-lipoic acid (102.86 μg/g diet), vitamin E succinate (8.57 μg/g diet) with or without coenzyme Q10 (51.43 μg/g diet) (275, 276). These results indicate that BBIC, SeM and the antioxidant combinations are potentially useful as countermeasures against space radiation-induced oxidative stress and subsequent adverse biological effects, which could arise from the increased oxidative stress in irradiated subjects.

5.2. Antioxidant Protection against Radiation Induced Cell Death and Transformation *In Vitro*

The protective effects of the antioxidants, BBI and BBIC against radiation induced cell death have been evaluated *in vitro* using the clonogenic survival of cultured MCF10 cells or HTori-3 cells as the biological endpoints (272, 277). Irradiation with 5-GeV/n ^{56}Fe ions resulted in a dose-dependent decrease in the clonogenic survival of the MCF10 cells, which was attenuated by treatment with SeM alone or in combination with ascorbic acid, coenzyme Q10 and vitamin E succinate with an estimated dose modifying factor (DMF) of 1.2, 1.4, 2.2 and 2.2, respectively, for BBI, BBIC, SeM alone or in combination with other antioxidants (277). The identical DMF values for treatments with SeM alone or in combination with other antioxidants indicate that these antioxidant combinations were not more effective than the SeM treatment alone under the experimental conditions utilized. These results do not rule out the possibility that the antioxidant combinations might have

provided better protection than SeM treatment alone under other experimental conditions or for other biological endpoints, however, since a combination of antioxidants with different biochemical properties and action mechanisms is likely to provide better protection for different molecular targets during and after irradiation. For examples, cationic thiols are much more effective than anionic thiols in protecting DNA against radiation damage (278, 279), and lipophilic antioxidants, such as vitamin E, are effective protectors of biomembranes (248, 280), whereas hydrophilic antioxidants are more effective in protecting soluble proteins and enzymes in the aqueous environment of cells (281).

The protective effects of the antioxidants, BBI and BBIC, against proton and HZE particle radiation induced cell transformation were determined in HTori-3 cells by the soft agar colony formation assay, which measures the capability of HTori-3 cells to grow in an anchorage-independent manner. The results indicate that treatment of the cells with BBI, BBIC, SeM alone or in combination with ascorbic acid, coenzyme Q10 and vitamin E succinate prevented proton and HZE particle radiation induced HTori-3 cell transformation *in vitro* (272, 277). In experiments performed with γ-ray radiation, treatment with 5 μM SeM before, during and/or as late as 7 days after the radiation exposure brought the anchorage-independent colony formation efficiency down to levels that were not significantly different from the sham radiation controls (282).

5.3. Antioxidant Protection against Space Radiation Induced Mortality

It is well known that the hematopoietic system is highly sensitive to total body irradiation (TBI) and the fate of hematopoietic cells after TBI may determine the survival or death of irradiated subjects (283, 284). Thus, we have evaluated the effects of antioxidants in mice using the 30-day survival level and hematopoietic cell counts as the biological endpoints. Dietary supplementation with an antioxidant combination consisting of SeM (0.06 μg/g diet), α-lipoic acid (85.7 μg/g diet), NAC (171.4 μg/g diet), sodium ascorbate (142.8 μg/g diet) and vitamin E succinate (71.4 μg/g diet) significantly improved the 30-day survival of the mice irradiated with 8 Gy of X-rays (132) or 5.9 Gy of protons (139). However, no significant improvement was observed for the mice irradiated with protons at higher radiation doses (6.8 or 7.2 Gy) (139), It is expected that the higher proton radiation doses used in these studies might have caused damage that was beyond mitigation by the antioxidant treatment. In both the proton and X-ray radiation experiments, antioxidants were more protective when the antioxidant treatment was initiated 2 hours after radiation exposure as compared to the antioxidant treatment initiated 7 days prior to radiation exposure (132, 139). These results were confirmed by other investigators, who also showed that the antioxidant combination had a better protective effect on radiation induced lethality when started 7 days post-irradiation than it did when it was started 2 hours post-irradiation (285). The findings that antioxidant treatment leads to better survival when applied post-irradiation might result from an adaptive response to radiation exposure, which has been reviewed recently (286, 287). The ability of the antioxidants to improve radiation survival even when the antioxidant treatment was initiated after the radiation exposure suggests that antioxidants can be a feasible countermeasure for radiation exposure associated with space travel, radiation accidents or terrorist attacks in which radiation exposure could occur without much advanced warning.

The antioxidant treatment significantly attenuated the radiation effects on peripheral hematopoietic cell counts in the mice irradiated with 1 or 8 Gy of X-rays (132) or 1 or 7.2 Gy of 1-GeV protons (139). The antioxidant treatment was also shown to improve the recovery of the bone marrow cell counts in mice irradiated with γ-rays (132) or 1-GeV protons (139). Thus, antioxidants appear to be effective for protection of hematopoietic cells against the adverse effects of either photon or proton radiation. The ability of antioxidants to prevent the radiation caused loss of circulating neutrophils, also called PMNs, are illustrated in Figure 8, which shows that the mice maintained on an antioxidant diet and exposed to an 8 Gy dose of radiation experienced a drop in the levels of PMNs/neutrophils of a magnitude comparable to the effects observed for mice exposed to a 1 Gy dose of radiation and maintained on the normal diets. The mice maintained on the antioxidant diets and exposed to a 1 Gy dose of radiation had PMN/neutrophil cell counts that were not significantly different from those in the control animals. Thus, it is concluded that the antioxidant diet had highly significant beneficial effects on PMN/neutrophil cell counts in irradiated animals.

5.4. Antioxidant Protection against Space Radiation Induced Cataracts

An increased rate of cataract formation has been observed in astronauts (103, 104), which has been attributed to the increased exposure to cosmic radiation during space travel. To investigate the ability of BBIC and antioxidants to reduce the formation and severity of cataracts related to space radiation exposure, mice were exposed to 1-GeV proton or 1-GeV/n ^{56}Fe ion radiation and fed with a control diet or diets supplemented with BBIC or the antioxidant combination containing SeM, NAC, ascorbic acid, coenzyme Q10, α-lipoic acid and vitamin E succinate before and for 2 years after the radiation exposure (226). Lenses were harvested approximately 2 years after the radiation exposure for evaluation of the lens opacifications. The results showed that treatment with BBIC or the antioxidant combination decreased the prevalence and severity of the lens opacifications in the mice irradiated with 3 Gy of 1-GeV proton or 0.5 Gy of 1-GeV/n ^{56}Fe ion radiation, although statistical significance was only achieved for the ^{56}Fe ion irradiated mice, possibly due to the higher proton radiation dose (3 Gy) that might have exceeded the protective capacity of the antioxidant combination or BBIC. These results indicate that BBIC and the antioxidant combination could be useful for protecting astronauts against space radiation-induced cataracts during or after long-term manned space missions.

5.5. Antioxidant Prevention of Space Radiation Induced Cancer

Radiation induced malignancy is a particularly important risk associated with extended space travel. In a 2-year study performed with CBA/JCR HSD mice irradiated with 3 Gy of 1-GeV protons or 0.5 Gy of 1-GeV/n ^{56}Fe ions, treatment with the antioxidant combination or BBIC decreased the fractions of animals with malignant lymphoma to levels that were not significantly different from the baseline level (73). The treatment with the antioxidant combination or BBIC also prevented the increase in the fractions of mice with premalignant or malignant lesions of myeloid origin after the proton irradiation and the incidence rate of rare tumors (which included Harderian gland tumors) after the proton or ^{56}Fe ion radiation exposures (73, 225). From these experiments, it was concluded that antioxidants have a major protective effect against space radiation induced carcinogenesis *in vivo* (225). In these studies, a major protective effect resulted from the ability of the antioxidants to prevent the

early stage neoplastic growths from growing into fully developed, malignant tumors. Other studies suggest that anticarcinogenic agents can be added at late times following carcinogen exposure, in both *in vitro* and *in vivo* systems, and still have a suppressive effect on the carcinogenic process (261). The results of the studies on radiation induced carcinogenesis suggest that antioxidant and BBIC supplements could be useful for the prevention of malignancies and other neoplastic lesions developing as a result of exposure to space radiation. It has been concluded from these studies that antioxidants have a major protective effect against radiation induced carcinogenesis, and are effective even when added at late times during the carcinogenic process.

5.6. Mechanism(s) for Antioxidants as Radiation Countermeasures

To study the mechanisms for the radioprotection by the antioxidant treatment, we have examined the expression of the ATR gene, which is one of the central components of the DNA damage response pathway (288), and the CHK2 gene, which is a cell cycle checkpoint regulator and putative tumor suppressor (289, 290), in HTori-3 cells irradiated with 0.4 Gy of 5-GeV/n ^{56}Fe ions with or without SeM (5 μM) treatment initiated 24 hours prior to the radiation exposure. The results indicate that the ATR mRNA level was increased by 42% with the SeM treatment alone and increased by 94% with the SeM treatment and radiation exposure (272) In the same study, SeM treatment alone did not significantly affect the CHK2 mRNA level, but the combined treatment with SeM and ^{56}Fe ion radiation increased the level of CHK2 mRNA by 99%. The up-regulation of ATR and CHK2 gene expression observed in the irradiated HTori-3 cells may prevent the cells from going through mitosis until the damage is repaired, thereby preventing the radiation damage from being fixed and leading to mutations and/or malignant transformation.

In ICR mice irradiated with 6.4 Gy of 1-GeV protons or 7.0 Gy of γ-rays, 15 genes belonging to the class of “apoptosis regulator activity” were differentially expressed in the spleen of mice fed an antioxidant supplemented diet as compared with mice fed with the control diet, and the antioxidant treatment inhibited apoptosis in the white pulp of the spleen following γ-ray irradiation, possibly by altering IL-6 signaling and by blocking the expression of the prokineticin PROK2, the ligand to the G protein-coupled receptors PROKR1 and PROKR2 (233), which are involved in a number of pathophysiological processes. In ICR mice irradiated with 1 or 8 Gy of X-rays, bcl-2 gene expression was found to be decreased, whereas bax, caspase 7, caspase 9 and TGF-β1 gene expression was increased at 4 or 24 hours after irradiation. Dietary supplementation with antioxidants attenuated the radiation effects on bax, caspase 7, caspase 9 and TGF-β1 gene expression, but increased bcl-2 gene expression by 10 fold at 24 hours after irradiation (132). The abrogated pro-apoptosis (bax and caspase 9) gene expression and the increased anti-apoptosis (bcl-2) gene expression observed in the irradiated animals treated with antioxidants have implicated apoptosis as a key process modulated by antioxidants to attenuate the effects of radiation on the hematopoietic system and animal survival (132). The anti-apoptotic effects of selenium have also been reported in normal human or mouse fibroblasts (291) and primary human keratinocytes (292) irradiated with ultraviolet irradiation. The anti-apoptotic effect of selenium observed in normal animals or tissues after irradiation is in contrast to the pro-apoptotic effect of selenium observed in malignant cell

lines or tissues (293–295). The differential effects of selenium on apoptosis in normal and malignant cells/tissues suggest the possibility that selenium may protect normal tissues against radiation damage without the unintended consequence of suppressing radiation induced apoptosis in malignant cells or tissues. The different effects of antioxidants in normal cells, as compared to their effects in malignant cells, or those in different stages of carcinogenesis, have been discussed elsewhere (296).

In a separate study performed with cultured HTori-3 cells exposed to low doses (0.1 and 0.2 Gy) of 1-GeV/n ^{56}Fe ion radiation, treatment with 5 μM SeM in the medium for 24 hours prior to irradiation profoundly affected the radiation induced alterations in gene expression (297). The exposure to 0.1 and 0.2 Gy of ^{56}Fe ion radiation induced significant differential expression of 196 and 610 genes, respectively, and the differential expression of 39% to 55% of these genes was abolished by the SeM treatment (298). Genes and functional pathways that were significantly up- or down-regulated by the ^{56}Fe ion irradiation in the absence, but not in presence, of SeM treatment have been summarized previously (297, 298). Of particular interest was a cluster of chemokine and cytokine genes, e.g., CXCL1, CXCL2, IL6, IL11, IL8, IL24 and TGFβ2, which showed increased expression after irradiation with 0.1 Gy of 1-GeV/n ^{56}Fe ions in the absence, but not in the presence, of 5 μM SeM in the medium before and during the radiation exposure (299). It is also noteworthy that SeM has been shown to reduce space radiation induced effects by mitigating stress-related signaling pathways and downregulating certain genes associated with cell adhesion (111). These results suggest that SeM is potentially useful as a countermeasure to prevent some of the acute inflammatory/immune responses induced by low-dose HZE particle radiation.

5.7. Granulocyte Colony-Stimulating Factors as Countermeasures

In addition to the antioxidants, two forms of granulocyte colony-stimulating factors (G-CSFs), filgrastim and pegfilgrastim, were evaluated as countermeasures using the neutrophil count in ICR mice irradiated with γ-rays or SPE-like protons as the biological endpoint. The results demonstrated that exposure to SPE-like proton radiation or γ-ray radiation at doses up to 2 Gy significantly decreased circulating neutrophil counts in a dose and time dependent manner, which was prevented by treatment with either form of G-CSF evaluated in the study (135). These results indicate that both forms of G-CSFs could be a potential countermeasure for the reduced number of neutrophils in irradiated animals, although pegfilgrastim appears to be superior since its stimulatory effect on the neutrophil count was more pronounced and lasted longer than that of filgrastim.

In mouse studies using G-CSF (Neulasta) as a countermeasure for bacterial challenge toxicity in animals exposed to SPE radiation along with HS, Neulasta treatment was shown to reduce morbidity from 80–90% to 20–30% in γ-ray or proton irradiated C3H/HeN mice exposed to HS and challenged with *Pseudomonas aeruginosa* bacteria (Drew Weissman and colleagues, Unpublished data). In an experiment performed with ferrets (aged 12–15 weeks), that were exposed to a 2 Gy total body dose of γ-ray radiation with subcutaneous injection of phosphate buffered saline (PBS) or Neulasta (0.1 mg/kg) on days 1, 4, and 7 post-irradiation, peripheral blood was collected from each animal on day 0 (prior to radiation

exposure), and on days 1, 4, 7, and 13 post-irradiation for analysis. The results demonstrate that the Neulasta treatment of irradiated ferrets could lead to a significant increase in neutrophil counts (*Krigsfeld, G.S., Sanzari, J.K. and Kennedy, A.R., Unpublished data*). In a similar experiment performed with pigs (aged 12–15 weeks) that were exposed to a 2 Gy total body dose of SPE-like proton radiation and subcutaneous injections of PBS or Neulasta (0.1 mg/kg) on days 4, 7, and 10 post-irradiation, peripheral blood was collected from each animal on day 0 (prior to radiation exposure), and on days 1, 7, 10, 13, and 30 post-radiation for analysis. The results indicated that the proton radiation exposure led to statistically significant decreases in neutrophil counts at days 7, 10 and 13 post-irradiation in the irradiated animals injected with PBS; however, in irradiated pigs treated with Neulasta, the neutrophil counts were never decreased to below the baseline levels (*Sanzari, J.K., Krigsfeld, G.S., Shuman, A.L. and Kennedy, A.R., Unpublished data*). The overall conclusion from the studies in mice, ferrets and pigs is that Neulasta can increase the number of circulating neutrophils in three different species of animals evaluated.

5.8. SI-Wu-Tang or Fructose as Countermeasure to Increase Neutrophil Counts in Mice Exposed to SPE or γ-ray Radiation

A Chinese formula SI-Wu-Tang (SWT, 四物汤) is currently given to radiation and chemotherapy cancer patients in China to mitigate the adverse effects of cancer therapy on the numbers of circulating blood cells. One of the major ingredients in SI-Wu-Tang is fructose, as reviewed (235). We have performed studies in mice to determine whether these compounds in traditional Chinese medicine were able to mitigate reduced circulating blood cell counts following γ-ray or SPE-like proton radiation. The main conclusions of the studies were as follows: 1) SWT and fructose were both capable of increasing the number of circulating lymphocytes in γ-ray or SPE proton irradiated mice, and 2) fructose was more effective than SWT in increasing the number of circulating lymphocytes in γ-ray or proton irradiated mice (235). These results are important since there are essentially no countermeasures for lymphocyte loss following radiation exposure. Lymphocytes are among the most sensitive cells in the body to radiation exposure (141, 143, 144, 300), and a reduced lymphocyte count in the circulation at post-irradiation times is expected to result in greater susceptibility to infections. The fact that both SWT and fructose can be given orally makes them attractive for use during space travel.

5.9. Antibiotics as Countermeasures for Bacterial Toxicity in Mice Exposed to SPE Radiation and HS

Enrofloxacin, an antibiotic for veterinary use, was studied for its effectiveness as a countermeasure in experiments using the bacterial challenge model in mice. Enrofloxacin is available in an oral form but the mice in these experiments were treated with subcutaneous drug. Control, irradiated (2 Gy), hindlimb suspended, and irradiated and hindlimb suspended mice were treated with *Pseudomonas aeruginosa* bacteria at a dose that can be cleared by untreated animals or, in later experiments, with a dose of bacteria that leads to morbidity in approximately 50% of untreated mice. With both challenge doses, the institution of enrofloxacin at the time of bacterial challenge reduced morbidity from 80–100% to 0%. This demonstrated the complete effectiveness of prophylactic antibiotic treatment in the

protection of irradiated and hindlimb suspended mice against bacterial toxicity with the potential to lead to morbidity/death (Drew Weissman, unpublished data).

While the effectiveness of the antibiotic countermeasure cannot be surpassed, it does carry a number of potential adverse effects, especially, if it will need to be used multiple times, including the generation of antibiotic resistant bacteria. Thus, it is believed that alternative countermeasures for bacterial toxicity, with different mechanisms of action than those of antibiotics, will be useful for space travel in future exploration class missions.

5.10. Countermeasures for Radiation Induced Emesis in Ferrets

In studies performed with ferrets, 5- HT3 receptor antagonists, such as Zofran, have been shown to prevent or ameliorate vomiting and retching in ferrets following proton radiation exposure (301). As Zofran is maintained on the ISS for nausea and vomiting, it is the recommended 5-HT3 receptor antagonist to prevent or mitigate radiation induced nausea and vomiting during space travel.

5.11. Countermeasures for Altered Bleeding Times in Ferrets after SPE Radiation Exposure

It is well established that endotoxin (lipid A portion) released by Gram negative bacteria activates certain factors of the intrinsic coagulation cascade, such as Factor XII, which in turn initiates fibrin formation and increases PT/aPTT values, thereby increasing the risk of DIC development. To counteract the factor deficiencies, BeneFIX (recombinant Factor IX) was evaluated as a countermeasure for the proton radiation induced coagulation cascade. Treatment with BeneFIX reduced the clotting times in irradiated ferrets back to levels that were essentially equivalent to those of the non-irradiated control ferrets (208). Since treatment with BeneFIX increases the concentration of Factor IX, a factor depleted post-irradiation, BeneFIX could have beneficial effects on coagulation when administered after the radiation exposure.

Phytonadione (vitamin K) is essential for post-translational modification of a glutamate to a carboxylated-glutamate that is necessary for Factor II, VII, IX, and X (199). It was also evaluated as a countermeasure for activation of the proton radiation induced coagulation cascade. The results indicate that phytonadione had a minor beneficial effect on PT values in the proton irradiated ferrets, but did not affect the aPTT values (208).

Treatment of DIC with blood clotting factors or other components of plasma is a topic that has been reviewed previously (302). We have demonstrated that SPE-like proton radiation led to hypocoagulability by activating the clotting cascade that consumes factors involved in coagulation. SPE-like proton radiation can also cause leucopenia and severe lymphocytopenia, which, in combination with the effects of the SPE-proton radiation on hemostasis, could have major health consequences in irradiated subjects. Our studies have shown that BeneFIX can serve as a potential countermeasure for the increased bleeding times in ferrets caused by exposure to SPE-like proton radiation.

5.12. Corticosteroid as a Countermeasure for Radiation Induced Pneumonitis and Pneumonopathy in Pigs

Several pigs exposed to simulated SPE radiation developed symptomatic, radiation-associated pneumonopathy that radiographically involved all lung fields but was worse in the pleura and apices where the radiation dose was found to be highest (130); many of the pigs with this condition developed nonproductive coughs. Thoracic radiographs and diagnostic CT scans were performed, and the CT findings were consistent with an acute lung injury concurrent with chronic bronchial changes and volume loss. Differential diagnoses for these findings were radiation induced lung injury or atypical infectious bronchopneumonia. To help differentiate between these possibilities, sequential antibiotic therapy, which included atypical (e.g., mycoplasma), Gram-positive and Gram-negative coverage, was initiated. After two weeks of therapy, it was concluded that antibiotics were not effective treatments for the condition. Corticosteroid therapy then began and the pig symptoms improved rapidly, with dramatic improvement and resolution of imaging abnormalities observed within one month. It was concluded that corticosteroids were effective treatments for the pneumonopathy and/or pneumonitis that developed in the pigs exposed to SPE-like radiation (130).

5.13. Mometasone as a Countermeasure for SPE Proton Radiation Induced Skin Lesions in Pigs

Pigs were exposed to either a 5 Gy or 10 Gy dose of SPE-like electron irradiation, and seven types of creams were applied to the trunk of the animal in 1 $inch^2$ patches covered with Tegaderm dressing consecutively for 14 days immediately after the radiation exposure. The only cream that appeared to mitigate the radiation-induced initial hyperpigmentation was the cream that contained corticosteroids (mometasone cream [Elecon]), as compared to the other creams, which were water-based emollients, a platelet growth factor containing cream, a triple antibiotic containing cream, or the untreated area that was not exposed to any cream other than the Tegaderm dressing. At 14 days post-irradiation, biopsies were performed that revealed decreased melanosomes, necrotic keratinocytes and melanin deposition in the areas of irradiated skin treated with mometasone compared to the untreated irradiated skin. Thus, it was concluded from these studies that topical application of steroids mitigate skin toxicity produced by exposure to SPE-like proton radiation (*Cengel, K.A. and Sanzari, J.K., Unpublished data*).

5.14. Transparent Film Dressing for Protection against Proton Therapy Induced Skin Reactions in Humans

Patients undergoing proton therapy for prostate cancer frequently develop radiation dermatitis. It has been reported that two prostate cancer patients undergoing proton cancer therapy at the University of Pennsylvania had radiation dermatitis that appears to have been substantially diminished by the presence of transparent film dressings (Beekley stickers) (234). In these studies, small circular (2.5 cm diameter) transparent adhesive markers were placed on their skin to assist with daily alignment in these patients treated with a total proton dose of 79.2 Gy in 1.8 Gy fractions, using two opposed lateral beams daily. It was observed that the covered areas of the skin exhibited considerably diminished radiation dermatitis

compared to the uncovered areas of the skin, and this difference persisted for at least one month after the therapy period ended. A phantom dosimetric study was performed to evaluate the impact of the transparent film dressing on a beam's SOBP, and the results indicated no gross dosimetric effect. Thus, the transparent adhesive markers appear to have attenuated radiation dermatitis in these two patients without affecting the SOBP. It is hoped that this finding can improve proton-related radiation dermatitis in other types of treatments for cancer as well (i.e., in other conditions in which proton radiation exposure could lead to radiation dermatitis).

6. Discussion

In recent years, we have been engaged in *in vitro* and animal studies on acute biological effects of the types of radiation at the energies, doses and dose-rates relevant to space travel. Three species of animals, i.e., ferrets, pigs and mice, were used in our studies to evaluate the effects of radiation on various biological endpoints including survival, cancer development, hematopoietic cell counts, emesis, blood coagulation, CNS endpoints (which included social exploration, submaximal exercise treadmill, and locomotor activity), cataract development, oxidative stress, gastrointestinal tract bacterial translocation, immune activation, and gene expression associated with programmed cell death and ECM remodeling.

For 30-day survival, 1-GeV/n proton radiation appeared to be more lethal than X-rays to mice (132, 139), but SPE-like proton radiation was comparable to γ-rays for ferrets (142). Ferrets are considerably more sensitive than mice to the lethal effects of radiation exposure. In experiments with mice irradiated at lower doses of protons (3 Gy) or HZE particles (0.5 Gy), acute effects of the radiation doses were not observed over a 30 day experimental period but the long-term survival in the irradiated mice was reduced significantly by the radiation exposure, and the decreased long-term survival was accompanied by a significant increase in the rate of malignant lymphoma and Harderian gland tumors, as well as the fractions of animals with malignant lymphoma or rare tumors (73, 225).

With respect to peripheral WBC and lymphocyte counts, the effects of proton irradiation given as a homogeneous dose to the mice is not affected by the dose fractionation, dose-rates or proton energy in the ranges evaluated. In contrast, simulated hypogravity brought about by PWS was shown to potentiate splenic lymphocytes to the cell killing effects of radiation (136). A number of other studies indicated that SPE radiation and simulated microgravity produced by HS led to synergistic adverse effects on hematopoietic and immune cell functions, including bacterial containment in the GI tract (152), T-cell activation (136, 138), and death from a bacterial challenge at a sub-lethal dose (151).

For emesis in ferrets, the risk of SPE radiation-induced vomiting is low and may reach statistical significance only when the radiation dose reaches 1 Gy or higher. The ED_{10} and ED_{50} values estimated for the fraction of animals that retched or vomited after proton irradiation at the high dose rate (0.5 Gy/minute) were lower than the lower limits of the respective 95% confidence intervals established for γ-rays, suggesting that high dose rate proton irradiation was more effective than high dose rate γ-ray irradiation in inducing retching and vomiting. There was a large sparing effect observed at the low dose rates

expected for SPE radiation, such that the results for vomiting and retching in response to low dose-rate exposure to SPE radiation were not statistically significant when compared to control animals. The trend, however, in the experiments performed at the low dose rate appeared to indicate more retching and vomiting episodes in ferrets irradiated with protons than in the animals exposed to γ-rays at the same dose and dose rate (194).

The results from some of the studies described here indicated that proton radiation had considerably more severe adverse effects compared to those produced by comparable doses of the reference radiations used, which include x-rays, γ-rays and electrons. These include studies on mouse survival after irradiation with protons and x-rays (132, 139), on ferret hematopoietic cell counts after exposure to SPE protons and γ-rays (141), on ferret retching and vomiting induced by SPE protons and γ-rays (194), on blood clotting times in ferrets exposed to SPE protons and γ-radiation (205) and on peripheral hematopoietic cell (WBC, lymphocyte and neutrophil) counts in minipigs exposed to simulated proton SPE or simulated electron SPE radiation (6 + 12 MeV electrons), which resulted in a comparable dose distribution to the proton SPE radiation expected in an SPE), especially at the low end of the radiation dose range evaluated (144). The results of other studies described here suggest that the effects of proton radiation were comparable to those of the reference radiations evaluated. These studies include studies on the lack bacterial containment in the GI tract from exposure to γ-rays or SPE proton radiation in mice (153), on fatigue in mice after irradiation with γ-rays or protons (211, 213), on the skin changes resulting from exposure to SPE proton or electron radiation (130), on the development of DIC in irradiated ferrets (142) and on mortality of mice exposed to radiation alone or radiation with exposure to microgravity, along with a bacterial challenge (Drew Weissman and colleagues, Unpublished data). Overall, the differences in the effects of the proton and reference radiation were relatively small or limited to the lower end of dose range (~0.5 Gy) evaluated.

The mechanism(s) for mortality in irradiated mammals is not well understood, although numerous hypotheses have been proposed. At the lowest total body radiation doses leading to mortality, death occurs from the hematopoietic syndrome, which is believed to result from the cell killing effects of radiation in the bone marrow that lead to low numbers of circulating blood cells and the resultant hematopoietic symptoms, such as infection and bleeding due to the loss of leukocytes and platelets. Over the last half century, the radiation dose required to kill half of the irradiated subjects, known as the LD_{50}, has been used as a parameter of radiation sensitivity for comparisons among various mammalian species. It is well-known that the LD_{50} value is highly variable for different mammalian species; however, the bone marrow cell sensitivity to ionizing radiation is remarkably similar among different species, strains and individuals (303, 304). These results suggest that the lethal effects of radiation on bone marrow and hematopoietic cells may not be the primary mechanism for radiation exposure related death. Our studies in ferrets irradiated with SPE-like protons have suggested that radiation induced activation of the coagulation cascade may result in DIC, which could be a major mechanism by which relatively low doses of radiation lead to mortality (142). The human LD_{50} values for radiation induced death are imprecise because there have been relatively few cases in which human subjects were exposed to radiation at doses near the LD_{50}, especially in the last half century. The estimated human

LD_{50} value for ionizing radiation ranges from 3 to 4 Gy for young adults, without medical intervention to 2 to 3 Gy for the very young or the old (305). Remarkably different LD_{50} values have been reported for different species (304–306) with ferrets as the most sensitive mammalian species (142, 306), closely followed by dogs and pigs (304). The LD_{50} in Gottingen pigs is as low as 1.8 Gy and widespread hemorrhaging is observed at death of the irradiated Gottingen pigs (307), with some evidence of DIC at doses near the LD_{50}, such as the rapid onset of systemic inflammation (C-reactive protein, fibrinogen) and multi-organ dysfunction (308). Similarly, dogs exhibit hemorrhagic diathesis at doses near the LD_{50}, and die with signs resembling DIC (309, 310). While DIC has not been diagnosed as a cause of radiation induced death in the pig or dog studies or in irradiated human populations, a hallmark of DIC, i.e. hemorrhage, at death has been frequently observed in irradiated mammals, including humans. There is extensive evidence that widespread hemorrhages occurred in the Hiroshima and Nagasaki atomic bomb casualties, even in the relatively low radiation dose groups (311), with the estimated LD_{50} values of approximately 2.5 (312, 313). Other information about hemorrhaging in humans after irradiation comes from accidental exposures in Norway (314) and Brazil (315, 316) in which several people were accidently exposed to whole body irradiation at doses near the human LD_{50} and widespread hemorrhages were observed.

A reduction in the number of platelets can result in hemorrhaging and death; however, our studies suggest that the blood clotting abnormalities in irradiated ferrets is not caused by a reduction in the number of platelets (205, 208). A similar phenomenon has been reported in irradiated dogs in which the platelet counts are not depressed in dogs at a “preterminal” stage in which bleeding abnormalities are observed, although the platelet counts are decreased greatly in irradiated dogs with full-blown DIC (310). In atom bomb casualties, widespread hemorrhages were observed in people before the level of platelet counts had fallen to levels expected to cause hemorrhage (311). Furthermore, while platelet injections and other blood clotting factors can have beneficial effects on hemorrhaging, platelet infusions do not prevent all deaths from the radiation exposure (317).

Hemorrhaging and signs of DIC have been frequently reported in higher mammalian species, such as dogs and pigs; however, hemorrhaging has not been reported in mice at doses near the LD_{50}, which is the species that is most often used in radiobiology studies. Bacteremia is the leading cause of death in mice (317, 318), whereas hemorrhage is thought to be the major cause of death in dogs, rabbits, guinea pigs (317) or pigs (319) after irradiation at doses near the LD_{50} dose for each species. The pathophysiology of the hematopoietic syndrome in pigs is thought to be similar to that observed in humans (307). Such differences suggest that we need new ways of thinking about mechanisms for death after irradiation at doses near the LD_{50} level. Since the human LD_{50} is closer to the LD_{50} of ferrets, dogs and pigs than to the LD_{50} of mice, species such as ferrets, dogs and pigs are likely to be better animal models than mice for evaluating the effects of radiation and radiation countermeasures on bleeding, coagulation cascade activation and DIC risks for humans.

It is noteworthy that the LD_{50} values for these larger mammals are considerably lower than those observed in various strains of mice (304). Examples of some published LD_{50} values

for mammals are as follows: Ferrets – 1.5 Gy (142), Gottingen pigs – 1.8 Gy (307), Yucatan minipigs-2.5 Gy (Krigsfeld, G.S., Shah, J.B., Sanzari, J.K., Lin, L., Kennedy, A.R., Unpublished data), pigs – average value – 2.6 Gy (304), dogs – average value – 2.6 Gy (304), monkeys –5.07 Gy (320), mice – average value – 8.16 Gy (304). Using published data, we have compared the percentage of animals exhibiting hemorrhage at death (from exposure to radiation doses near the LD_{50} level) as a function of the LD_{50} of the species and observed an excellent correlation between the percent of animals developing "DIC" (from hemorrhaging at death, the hallmark of DIC) and the LD_{50} value for the species/strain (Krigsfeld, G.S. and Kennedy, A.R., Unpublished data). These figures for animal hemorrhaging at death range from 100% of ferrets and Yucatan pigs to 0% of the mice (as mice irradiated at radiation doses near their LD_{50} levels die from bacteremia (317, 318). Based on this analysis, it is hypothesized that the propensity to develop DIC determines the LD_{50} for the mammalian species. This hypothesis provides a novel and reasonable explanation for the great variability observed in LD_{50} values among different mammalian species.

There is a considerable amount of evidence that humans exhibit hemorrhaging at doses near the LD_{50}. The LD_{50} of the atom bomb casualties has been estimated to be approximately 2.5 Gy (312, 313). The percent of those dying from exposure to the atom bombs with evidence of hemorrhage can be estimated from the atom bomb casualty data, which has been reviewed by Liebow et al. (311). The casualty dose estimates are given in a report by the U.S. Atomic Energy Commission (321). As described by Liebow et al. (311), people exposed to radiation from the atom bomb used in Hiroshima have been classified into several groups. In Group II (patients dying during the third, fourth, fifth and sixth weeks or surviving with severe clinical symptoms), some of the people lived and some of the people died. It is expected that the people who died in Group II were those who received a dose of radiation near the human LD_{50} level. The data that were collected from each person represented one tissue section from each tissue/organ examined, and the fraction of tissues exhibiting hemorrhage is reported by Liebow et al. (311). For many of the organs/tissues (e.g., kidney, liver and other organs), the percentages exhibiting hemorrhage are as high as 60%, but it is not clear what fraction of exposed individuals experienced hemorrhaging in one or more organs. Therefore, it is assumed that 60% of the people exhibiting hemorrhage is a low estimate and that the true value lies between 60% and 100% of the people dying in Hiroshima (after exposure at a dose near the LD_{50}) had evidence of hemorrhage, which is similar to the high percentages observed for other large mammals (e.g., pigs and dogs).

It is also noteworthy that knowledge obtained about the adverse health effects of inhomogeneous doses of interventional and therapeutic radiation is relevant for evaluation of radiation effects in people resulting from exposure to interventional radiology procedures or therapeutic radiation, in which the skin doses are higher than the internal organ doses. An example of the relevance for patients on earth is that high doses of SPE-like radiation primarily to the skin with minimal to no significant doses to internal organs have been shown to produce adverse health effects in internal organs (e.g., pulmonary toxicities or pneumonitis with coughing in the lungs, bone marrow changes and reductions in circulating blood cell counts (130).

7. Summary Concerning the Major Effects of SPE and Space Radiation

7.1. Dosimetry

With the incorporation of modern radiation oncology approaches, such as CT based Monte Carlo dosimetry, into the Human Space Program, it has become feasible to accurately predict organ specific radiation doses for astronauts exposed to SPE radiation. Depending on the organ system of interest (deep vs. superficial) and the fluence/energy profile of the exposure (hard vs. soft event), either the physical size of the astronaut or the fluence/energy profile for the SPE can be the determining factor for radiation induced dose/toxicity.

7.2. Adverse Effects in Hematopoietic/Immune System Cells

Significant decreases in WBC counts were observed in mice and ferrets irradiated with SPE-like proton and γ-ray radiation at total body doses of 0.5 to 2 Gy, and in pigs irradiated with proton simulated SPE radiation at skin doses of 2 to 10 Gy and electron simulated SPE radiation at skin doses of 2.5 to 25 Gy. At the higher doses of proton or γ-ray radiation, the neutrophil counts in the blood of both mice and ferrets, but not pigs, reached critically low levels that, if observed in a patient in a hospital (e.g., following radiation or chemotherapy for cancer), would trigger a medical response and suggest the use of countermeasures to increase the level of neutrophils. In the pigs exposed to proton simulated SPE radiation, the neutrophil counts did not return to normal levels even at months after the radiation exposure.

7.3. Adverse Effects on the Immune System

At a dose of 2 Gy, SPE-like proton radiation or γ-ray radiation was shown to cause breaks in the epithelial layer of the small intestine of mice, which allows translocation of bacteria and bacterial products, such as LPS. The threshold for the morbidity/mortality effect in bacterial challenge studies was between 1.0 and 1.5 Gy. The proton radiation works synergistically with HS to result in an accumulation of a relatively large amount of LPS in the intervillous regions of the ileum. Due to the synergistic effect from HS, the threshold doses for immune system parameters determined without the HS treatment may underestimate the actual immune system risks for astronauts during space travel involving microgravity conditions.

Another major adverse effect observed in immune system cells is the lack of, or reduction in the level of, activation in T-lymphocytes in mice exposed to 1 – 2 Gy of SPE-like proton or γ-ray radiation, either with or without simulated microgravity (using the PWS and HS systems).

7.4. Emesis (Vomiting and Retching)

In female descented Fitch ferrets, irradiation with ^{60}Co γ-rays or 155-MeV protons at a high dose rate of 0.5 Gy/minute resulted in dose-dependent changes in the endpoints that are indicative of retching and vomiting. The minimum radiation doses required to induce statistically significant changes in retching- and vomiting-related endpoints were 0.75 and 1.0 Gy, respectively. The RBE of the proton radiation at the high dose rate (relative to the γ-rays at the same dose rate) did not differ significantly from 1. Similar, but smaller and less consistent, changes in the retching- and vomiting-related endpoints were also observed for ferrets irradiated with γ-rays and protons at the low dose rate of 0.5 Gy/hour. Since this low

dose rate is similar to a radiation dose rate expected during a SPE, these results suggest that the risk of SPE radiation-induced vomiting is low and is likely to reach statistical significance only when the radiation dose reaches 1 Gy or higher.

7.5. Skin Effects

At skin doses of 7.5 Gy and higher, there is a persistent immunological dysfunction in pig skin, which is characterized by an enhanced DTH response; a similar effect was observed in mice at doses of 2 Gy or less. At higher doses of radiation, the pig skin becomes more sensitive to touch (lymphedematous), and there can be blistering, burns and epithelial dysfunction (e.g., pigment incontinence, which indicates defective cell to cell transfer of biomolecules). The threshold radiation doses for the skin effects are 4.5 to 5 Gy. The decrease in vascular bed area is the most serious skin effect potentially resulting from irradiation at very high skin doses. However, this complication would be a highly unlikely occurrence for astronauts since such high radiation doses are not expected during most SPEs.

7.6. Disseminated Intravascular Coagulation

Both SPE-like proton and γ-ray radiation at doses near 2 Gy resulted in DIC in ferrets, which led to 100% mortality. The threshold dose of radiation for this effect is 1.5 Gy. This is an important finding, as DIC has not been established previously as a cause of death in mammals following radiation exposure. The blood clotting abnormalities in ferrets were observed at a very low dose (0.25 Gy, which was the lowest dose evaluated in the studies), and radiation at the SPE-like low dose rate resulted in more severe effects on the blood clotting parameters in ferrets than the high dose-rate irradiation.

Yucatan minipigs exposed to a 2.5 Gy dose of radiation were also diagnosed with DIC and died (or were euthanized). Minipigs exposed to a 2 Gy dose of SPE like proton radiation did not die, but they exhibited severe clotting abnormalities (increased bleeding times) that could be problematic during space travel.

Based on the work in ferrets and pigs performed as part of the space radiation studies described here, it is hypothesized that DIC may be a major cause of death in humans following exposure to relatively low doses of radiation.

7.7. Development of Vision Abnormalities

It was observed that pigs exposed to 2.5–7.5 Gy (skin dose) of simulated SPE - electron radiation (October 1972 event) exhibited increased opening pressure values, which lasted up to 90 days post-radiation. Other endpoints have also been evaluated which could have significance for the findings that many astronauts exposed to long-duration space flight have exhibited vision alterations. As one example, increased nerve sheath diameter has been observed in irradiated pigs exposed to SPE-like radiation (by ocular ultrasound examination). Increased nerve sheath diameters have also been documented by ocular ultrasound technology in astronauts and this finding has been implicated in the development of astronaut vision alterations (220).

A relatively low dose of SPE-like radiation (skin doses as low as 2.5 Gy) can lead to increased intracranial pressure in pigs, and a dose of 5 Gy of proton simulated SPE radiation was shown to increase the nerve sheath diameter. The statistically significant effects observed on these endpoints in the pig studies suggest that astronaut exposure to these relatively low doses of SPE radiation could exacerbate the vision alterations known to exist in astronauts during future exploration class missions.

7.8. Threshold Radiation Doses for Statistically Significant Adverse Biological Effects from SPE Like Radiation *In Vivo*

The estimated threshold radiation doses to cause significant adverse biological effects *in vivo* for various endpoints measured are summarized as follows:

- Reductions in WBC counts in mice and ferrets: a homogeneous dose of 0.5 Gy; in pigs: a homogeneous dose of 2.0 Gy (lowest dose evaluated) of SPE-like proton radiation.
- Levels of death in mice exposed to SPE like protons, along with HS, in response to a bacterial challenge: 1.0 to 1.5 Gy.
- Elevated levels of LPS and LPB in mouse serum: 2 Gy of SPE-like proton radiation.
- Increases in blood clotting time in ferrets: 0.25 Gy of SPE-like protons at a low, SPE-like dose rate.
- Lack of T cell activation in mice: 1 Gy.
- Skin effects: skin hyperpigmentation, epidermal thickening, and decrease in vascular bed area: 4.5 to 5 Gy of pSPE or eSPE.
- Emesis in ferrets (for high dose-rate irradiation): 1 and 0.75 Gy for vomiting and retching, respectively. For low SPE like dose-rates, the results were not statistically significant at doses up to 2 Gy for SPE like proton radiation compared to controls.
- Levels of death from, or signs of, DIC in ferrets or pigs. All ferrets died with signs of DIC at a dose of 2 Gy from SPE-like protons or γ-ray radiation. The threshold dose for this effect is 1.5 Gy. Ferrets are thought to be more susceptible to radiation induced DIC than humans, while the sensitivity of Yucatan minipigs to radiation induced DIC is thought to be like that of humans. In DIC studies performed in Yucatan minipigs, the pigs died from DIC at a dose of 2.5 Gy; 2.5 Gy is a potentially lethal dose of radiation for humans as well. At an SPE-like radiation dose of 2.0 Gy, pigs had severe clotting abnormalities suggestive of DIC. With the other space stressors (e.g., microgravity, elevated levels of oxygen during EVAs and carbon dioxide in the spacecraft, etc.), the lethal human dose may be considerably <2.5 Gy and astronauts may be susceptible to the onset and progression of DIC at doses that could be received from exposure to SPE radiation. In any case, astronauts are likely to have bleeding abnormalities from very low SPE radiation doses (25 cGy or below), and they should avoid exposure to anything that

could damage the skin integrity due to the bleeding risk following a sizeable exposure to SPE radiation.

7.9. RBE Values

A higher RBE value for a given effect indicates that a more severe effect is expected for exposure to SPE radiation than from conventional reference radiation (e.g., γ-rays, x-rays or electrons). In our studies, it was observed that there can be: 1) different RBEs for different biological endpoints in the same animal species/strain, and 2) different RBEs for the same biological endpoint in different species/strains. However, for most of the endpoints evaluated, which include hematopoietic blood cell counts, immune system parameters and fatigue measured in mice, emesis evaluated in ferrets, skin effects assessed in pigs, the RBE values were not significantly different from 1 except for the following:

- Hematopoietic blood cell counts in ferrets: RBE ranges from 1.2 to 1.6 for the WBC count at 48 hours after irradiation, RBE ranges from 1.9 to 2.1 for neutrophil count at 48 hours after irradiation (RBE values were particularly increased at the low end of the SPE proton dose range evaluated);
- Hematopoietic blood cell counts in pigs: RBE ranges from 2.4 to 4.1 for the WBC count, and 2.2 to 5.0 for the neutrophil count on day-4 post-irradiation (RBE values were particularly increased at the low end of the SPE proton dose range evaluated).

RBE values were determined for hematopoietic cell counts in mice, ferrets and pigs; therefore, they can be compared across these three species. From these data, it was observed that the RBE values of the SPE-like radiation for white blood cell counts vary greatly between mice, ferrets and pigs, with the RBE values being somewhat greater in ferrets than in mice, and considerably greater in pigs than in ferrets or mice. This trend suggests that the RBE values of SPE radiation for white blood cell counts could be considerably greater in humans than those observed in smaller mammals, and that SPE proton radiation may be far more hazardous to humans than previously estimated from studies performed in small animals (e.g., rodents).

7.10. Dose-rate Effects

One major endpoint evaluated that was affected by dose rate was ferret emesis, which was significantly increased after irradiation with SPE-like proton radiation or γ-ray radiation administered at the high dose rate of 0.5 Gy/minute, but not at a low dose rate of 0.5 Gy/hour. The other major endpoint affected by dose-rate was the blood clotting time, which was increased to a considerably greater extent in the studies with the low, SPE-like dose-rate than in the high dose-rate radiation studies. For all other endpoints, the sparing effects of the radiation dose rate are either insignificant or minimal and not biologically meaningful.

8. Agents Identified as Countermeasures for Space Radiation Induced Adverse Biological Effects

As a part of the work for this program, the following agents have been identified as countermeasures, which may lead to risk reductions in astronauts exposed to space radiations.

- **A.** Dietary antioxidants (132, 139), fructose (235) and G-CSFs (Neupogen, Neulasta) as countermeasures to prevent or alleviate the loss of circulating white blood cells (132, 139, 235), neutrophils (132, 135, 139), and lymphocytes (235).
- **B.** Orally administered antibiotics (e.g., enrofloxacin) as a countermeasure to prevent or alleviate translocation of bacteria and bacterial products as well as death from bacterial challenge in animals exposed to SPE-like proton radiation and hindlimb suspension [Drew Weissman, unpublished data]).
- **C.** 5-HT3 antagonists (e.g., oral ondansetron [Zofran]) as a countermeasure for proton radiation induced emesis (retching and vomiting) (196).
- **D.** Corticosteroid therapy as a countermeasure for pneumonopathy/pneumonitis that developed after radiation exposure (130).
- **E.** Topically applied steroid cream (mometasone-Elecon) (Cengel, K.A. and Sanzari, J.K., Unpublished data) and transparent film dressing (234) as countermeasures for radiation induced skin damage (e.g. hyperpigmentation).
- **F.** Dietary antioxidants as a countermeasure to decrease the risk of long-term radiation effects, e.g. cancer (73, 225) and cataracts (226).
- **G.** Benefix as a countermeasure for increased bleeding times after radiation exposure (208).

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Abbreviations

ARS	acute radiation sickness
aPTT	activated partial thromboplastin time
BBI	Bowman-Birk inhibitor
BBIC	BBI Concentrate
BFO	blood forming organs
BK	bradykinin
CNS	central nerve system
CT	computed tomography

DCF	dichlorofluorescein
DIC	disseminated intravascular coagulation
DMF	dose modifying factor
DNA-PKcs	DNA-dependent protein kinases
DTH	delayed type hypersensitivity
ECM	extracellular matrix
EGb76	quercetin
eSPE	simulated electron SPE
EVA	extra-vehicular activity
GI	gastrointestinal
GCR	galactic cosmic rays
G-CSF	granulocyte colony-stimulating factor
HDR	high dose rate
HS	hindlimb suspension
HZE particles	highly energetic, heavy, charged particles
ICRP	International Commission of Radiation Protection
IFN-α	interferon-alpha
INR	the patient's 'test' PT value divided by the laboratory 'normal' PT value, raised to the power of International Sensitivity Index
ISS	International Space Station
LAD	left anterior descending
LBP	lipopolysaccharide binding protein
LD_{50}	dose expected to kill 50% of the treated subjects
LDR	low dose rate
LET	linear energy transfer
LPS	lipopolysaccharide
MnSOD	manganese superoxide dismutase
NAC	N-acetyl cysteine
NASA	National Aeronautics and Space Administration
NCRP	National Council on Radiation Protection and Measurements
NK	Natural Killer
PAMP	pathogen associated molecular patterns

PBMNC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PHA	phytohemagglutinin
PMN	polymorphonuclear leukocyte
pSPE	simulated proton SPE
PT	prothrombin time
PWS	partial weight suspension
RBE	relative biological effectiveness
SCR	solar cosmic radiation
SEB	surrogate endpoint biomarker
SeM	L-selenomethionine
SOBP	spread out Bragg peak
SPE	solar particle event
SWT	SI-Wu-Tang
TAS	total antioxidant status
TBI	total body irradiation
TF	tissue factor
vWF	von Willenbrand factor
WBC	white blood cell

References

1. 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]
2. Katz R, Ackerson B, Homayoonfar M, Sharma SC. Inactivation of cells by heavy ion bombardment. Radiation Research. 1971; 47:402–425. [PubMed: 5561931]
3. 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. Radiation Research. 1999; 30:361–382.
4. Smart DF, Shea MA. The local time dependence of the anisotropic solar cosmic ray flux. Advances in Space Research. 2003; 32:109–114. [PubMed: 14727670]
5. NCRP, National Council on Radiation Protection and Measurements (NCRP). Guidance on radiation received in space activities. NCRP; 1989. Report No. 98.
6. NCRP, National Council on Radiation Protection and Measurements (NCRP). Information needed to make radiation protection recommendations for space missions beyond low-earth orbit. NCRP; 2006. Report No. 153.
7. Hu S, Kim M-HY, McClellan GE, Cucinotta FA. Modelling the acute health effects of astronauts from exposure to large solar particle events. Health Phys. 2009; 96:465–476. [PubMed: 19276707]
8. 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]

9. Sonnenfeld G, Mandel AD, Konstantinova IV, Berry WD, Taylor GR, Lesnyak AT, Fuchs BB, Rakhmilevich AL. Spaceflight alters immune cell function and distribution. J Appl Physiol. 1992; 73:191S–195S. [PubMed: 1526951]
10. Sonnenfeld G, Shearer WT. Immune function during space flight. Nutrition & Cancer. 2002; 18:899–903.
11. Sonnenfeld G. The immune system in space and microgravity. Medicine & Science in Sports & Exercise. 2002; 34:2021–2027. [PubMed: 12471311]
12. Sonnenfeld G. The immune system in space, including Earth-based benefits of space-based research. Current Pharmaceutical Biotechnology. 2005; 6:343–349. [PubMed: 16101473]
13. 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]
14. Uri JJ, Haven CP. Accomplishments in bioastronautics research aboard International Space Station. Acta Astronautica. 2005; 56:883–889. [PubMed: 15835037]
15. Setlow RB. The hazards of space travel. EMBO Rep. 2003; 4:1013–1016. [PubMed: 14593437]
16. 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 Sciences. 2006; 79:1317–1333. [PubMed: 16757003]
17. Kita M, Yamamoto T, Imanishi J, Fuse A. Influence of gravity changes induced by parabolic flight on cytokine production in mouse spleen. J Gravit Physiol. 2004; 11:67–68. [PubMed: 16145812]
18. Sastry KJ, Nehete PN, Savary CA. Impairment of antigen-specific cellular immune responses under simulated microgravity conditions. In Vitro Cell Dev Biol Anim. 2001; 37:203–208. [PubMed: 11409684]
19. Armstrong JW, Nelson KA, Simske SJ, Luttges MW, Iandolo JJ, Chapes SK. Skeletal unloading causes organ-specific changes in immune cell responses. J Appl Physiol. 1993; 75:2734–2739. [PubMed: 8125897]
20. Levine DS, Greenleaf JE. Immunosuppression during spaceflight deconditioning. Aviat Space Environ Med. 1998; 69:172–177. [PubMed: 9491259]
21. Crucian BE, Cubbage ML, Sams CF. Altered cytokine production by specific human peripheral blood cell subsets immediately following space flight. Journal of Interferon & Cytokine Research. 2000; 20:547–556. [PubMed: 10888111]
22. Aviles H, Belay T, Fountain K, Vance M, Sun B, 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]
23. Mehta SK, Stowe RP, Feiveson AH, Tyring SK, Pierson DL. Reactivation and shedding of cytomegalovirus in astronauts during spaceflight. J. Infect. Dis. 2000; 182:1761–1764. [PubMed: 11069250]
24. Lee EH, Ding W, Kulkarni AD, Granstein RD. Tumor growth and immune function in mice during hind-limb unloading. Aviat Space Environ Med. 2005; 76:536–540. [PubMed: 15945396]
25. Sonnenfeld G, Merigan TC. A regulatory role for interferon in immunity. Annals of the New York Academy of Sciences. 1979; 332:345–355. [PubMed: 231406]
26. Aviles H, O'Donnell P, Orshal J, Fujii H, Sun B, Sonnenfeld G. Active hexose correlated compound activates immune function to decrease bacterial load in a murine model of intramuscular infection. American Journal of Surgery. 2008; 195:537–545. [PubMed: 18304499]
27. Aviles H, O'Donnell P, Sun B, Sonnenfeld G. Active hexose correlated compound (AHCC) enhances resistance to infection in a mouse model of surgical wound infection. Surgical Infections. 2006; 7:527–535. [PubMed: 17233570]
28. Kennedy AR, Wan XS. Countermeasures for space radiation induced adverse biologic effects. Advances in Space Research. 2011; 48:1460–1479.
29. Stewart FA, Akleyev AV, Hauer-Jensen M, Hendry JH, Kleiman NJ, Macvittie TJ, Aleman BM, Edgar AB, Mabuchi K, et al. ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs--threshold doses for tissue reactions in a radiation protection context. Ann ICRP. 2012; 41:1–322. [PubMed: 22925378]

30. Cucinotta FA, Kim MH, Chappell LJ, Huff JL. How safe is safe enough? Radiation risk for a human mission to Mars. PLoS ONE. 2013; 8:e74988. [PubMed: 24146746]

31. Shapiro JR, Schneider V. Countermeasure development: future research targets. Journal of Gravitational Physiology. 2000; 7:1–4. [PubMed: 12124179]

32. Shapiro JR. Microgravity and drug effects on bone. Journal of Musculoskeletal Neuronal Interactions. 2006; 6:322–323. [PubMed: 17185807]

33. Schultheis L, Ruff CB, Rastogi S, Bloomfield S, Hogan HA, Fedarko N, Thierry-Palmer M, Ruiz J, Bauss F, Shapiro JR. Disuse bone loss in hindquarter suspended rats: partial weightbearing, exercise and ibandronate treatment as countermeasures. Journal of Gravitational Physiology. 2000; 7:13–14.

34. Morey-Holton ER, Globus RK. Hindlimb unloading of growing rats: a model for predicting skeletal changes during space flight. Bone. 1998; 5:83S–88S. [PubMed: 9600759]

35. Morey-Holton ER, Globus RK. Hindlimb unloading rodent model: technical aspects. Journal of Applied Physiology. 2002; 92:1367–1377. [PubMed: 11895999]

36. Morey-Holton E, Globus RK, Kaplansky A, Durnova G. The hindlimb unloading rat model: literature overview, technique update and comparison with space flight data. Advances in Space Biology & Medicine. 2005; 10:7–40. [PubMed: 16101103]

37. Alwood JS, Kumar A, Tran LH, Wang A, Limoli CL, Globus RK. Low-dose, ionizing radiation and age-related changes in skeletal microarchitecture. Journal of Aging Research. 2012; 2012:481983. [PubMed: 22570786]

38. Kondo H, Yumoto K, Alwood JS, Mojarrab R, Wang A, Almeida EA, Searby ND, Limoli CL, Globus RK. Oxidative stress and gamma radiation-induced cancellous bone loss with musculoskeletal disuse. Journal of Applied Physiology. 2010; 108:152–161. [PubMed: 19875718]

39. Lloyd SA, Bandstra ER, Willey JS, Riffle SE, Tirado-Lee L, Nelson GA, Pecaut MJ, Bateman TA. Effect of proton irradiation followed by hindlimb unloading on bone in mature mice: a model of long-duration spaceflight. Bone. 2012; 51:756–764. [PubMed: 22789684]

40. Yumoto K, Globus RK, Mojarrab R, Arakaki J, Wang A, Searby ND, Almeida EA, Limoli CL. Short-term effects of whole-body exposure to (56)fe ions in combination with musculoskeletal disuse on bone cells. Radiation Research. 2010; 173:494–504. [PubMed: 20334522]

41. Alwood JS, Yumoto K, Mojarrab R, Limoli CL, Almeida EA, Searby ND, Globus RK. Heavy ion irradiation and unloading effects on mouse lumbar vertebral microarchitecture, mechanical properties and tissue stresses. Bone. 2010; 47:248–255. [PubMed: 20466089]

42. Cherry JD, Liu B, Frost JL, Lemere CA, Williams JP, Olschowka JA, O'Banion MK. Galactic cosmic radiation leads to cognitive impairment and increased abeta plaque accumulation in a mouse model of Alzheimer's disease. PLoS ONE. 2012; 7:e53275. [PubMed: 23300905]

43. Davis CM, DeCicco-Skinner KL, Roma PG, Hienz RD. Individual Differences in Attentional Deficits and Dopaminergic Protein Levels Following Exposure to Proton Radiation. Radiation Research. 2014 **(in press)**.

44. Lonart G, Parris B, Johnson AM, Miles S, Sanford LD, Singletary SJ, Britten RA. Executive function in rats is impaired by low (20 cGy) doses of 1 GeV/u (56)Fe particles. Radiat Res. 2012; 178:289–294. [PubMed: 22880624]

45. Suman S, Rodriguez OC, Winters TA, Fornace AJ Jr, Albanese C, Datta K. Therapeutic and space radiation exposure of mouse brain causes impaired DNA repair response and premature senescence by chronic oxidant production. Aging (Albany NY). 2013; 5:607–622. [PubMed: 23928451]

46. Manda K, Anzai K, Kumari S, Bhatia AL. Melatonin attenuates radiation-induced learning deficit and brain oxidative stress in mice. Acta Neurobiologiae Experimentalis. 2007; 67:63–70. [PubMed: 17474322]

47. Manda K, Ueno M, Anzai K. Memory impairment, oxidative damage and apoptosis induced by space radiation: ameliorative potential of alpha-lipoic acid. Behavioural Brain Research. 2008; 187:387–395. [PubMed: 18006086]

48. Manda K, Ueno M, Anzai K. Space radiation-induced inhibition of neurogenesis in the hippocampal dentate gyrus and memory impairment in mice: ameliorative potential of the

melatonin metabolite, AFMK. Journal of Pineal Research. 2008; 45:430–438. [PubMed: 18631288]

49. Rivera PD, Shih HY, Leblanc JA, Cole MG, Amaral WZ, Mukherjee S, Zhang S, Lucero MJ, Decarolis NA, et al. Acute and fractionated exposure to high-LET $^{(56)}$Fe HZE-particle radiation both result in similar long-term deficits in adult hippocampal neurogenesis. Radiat Res. 2013; 180:658–667. [PubMed: 24320054]
50. Poulose SM, Bielinski DF, Carrihill-Knoll K, Rabin BM, Shukitt-Hale B. Exposure to ^{16}O-particle radiation causes aging-like decrements in rats through increased oxidative stress, inflammation and loss of autophagy. Radiat Res. 2011; 176:761–769. [PubMed: 21962006]
51. Tseng BP, Giedzinski E, Izadi A, Suarez T, Lan ML, Tran KK, Acharya MM, Nelson GA, Raber J, et al. Functional consequences of radiation-induced oxidative stress in cultured neural stem cells and the brain exposed to charged particle irradiation. Antioxid Redox Signal. 2013
52. Limoli CL, Giedzinski E, Baure J, Rola R, Fike JR. Redox changes induced in hippocampal precursor cells by heavy ion irradiation. Radiation & Environmental Biophysics. 2007; 46:167–172. [PubMed: 17103219]
53. Hienz RD, Brady JV, Gooden VL, Vazquez ME, Weed MR. Neurobehavioral effects of head-only gamma-radiation exposure in rats. Radiation Research. 2008; 170:292–298. [PubMed: 18763858]
54. Higuchi Y, Nelson GA, Vazquez M, Laskowitz DT, Slater JM, Pearlstein RD. Apolipoprotein E expression and behavioral toxicity of high charge, high energy (HZE) particle radiation. Journal of Radiation Research. 2002; 43:S219–S224. [PubMed: 12793762]
55. Rabin BM, Buhler LL, Joseph JA, Shukitt-Hale B, Jenkins DG. Effects of exposure to 56Fe particles or protons on fixed-ratio operant responding in rats. Journal of Radiation Research. 2002:S225–S228. [PubMed: 12793763]
56. Shukitt-Hale B, Casadesus G, McEwen JJ, Rabin BM, Joseph JA. Spatial learning and memory deficits induced by exposure to iron-56-particle radiation. Radiation Research. 2000; 154:28–33. [PubMed: 10856962]
57. Shukitt-Hale B, Carey AN, Jenkins D, Rabin BM, Joseph JA. Beneficial effects of fruit extracts on neuronal function and behavior in a rodent model of accelerated aging. Neurobiology of Aging. 2007; 28:1187–1194. [PubMed: 16837106]
58. Joseph JA, Erat S, Rabin BM. CNS effects of heavy particle irradiation in space: behavioral implications. Advances in Space Research. 1998; 22:209–216. [PubMed: 11541398]
59. Joseph JA, Shukitt-Hale B, McEwen J, Rabin BM. CNS-induced deficits of heavy particle irradiation in space: the aging connection. Advances in Space Research. 2000; 25:2057–2064. [PubMed: 11542857]
60. Rabin BM, Joseph JA, Shukitt-Hale B. Heavy particle irradiation, neurochemistry and behavior: thresholds, dose-response curves and recovery of function. Advances in Space Research. 2004; 33:1330–1333. [PubMed: 15803623]
61. Barkats M, Venault P, Christen Y, Cohen-Salmon C. Effect of long-term treatment with EGb 761 on age-dependent structural changes in the hippocampi of three inbred mouse strains. Life Sciences. 1995; 56:213–222. [PubMed: 7823780]
62. Bielefeldt-Ohmann H, Genik PC, Fallgren CM, Ullrich RL, Weil MM. Animal studies of charged particle-induced carcinogenesis. Health Phys. 2012; 103:568–576. [PubMed: 23032886]
63. Kennedy AR. Factors that modify radiation-induced carcinogenesis. Health Physics. 2009; 97:433–445. [PubMed: 19820453]
64. Shuryak I, Brenner DJ, Ullrich RL. Radiation-induced carcinogenesis: mechanistically based differences between gamma-rays and neutrons, and interactions with DMBA. PLoS ONE. 2011; 6:e28559. [PubMed: 22194850]
65. Weil MM, Bedford JS, Bielefeldt-Ohmann H, Ray FA, Genik PC, Ehrhart EJ, Fallgren CM, Hailu F, Battaglia CL, et al. Incidence of acute myeloid leukemia and hepatocellular carcinoma in mice irradiated with 1 GeV/nucleon (56)Fe ions. Radiat Res. 2009; 172:213–219. [PubMed: 19630525]
66. Trani D, Datta K, Doiron K, Kallakury B, Fornace AJ Jr. Enhanced intestinal tumor multiplicity and grade in vivo after HZE exposure: mouse models for space radiation risk estimates. Radiat Environ Biophys. 2010; 49:389–396. [PubMed: 20490531]

67. Dicello JF, Christian A, Cucinotta FA, Gridley DS, Kathirithamby R, Mann J, Markham AR, Moyers MF, Novak GR, et al. In vivo mammary tumourigenesis in the Sprague-Dawley rat and microdosimetric correlates. Physics in Medicine & Biology. 2004; 49:3817–3830. [PubMed: 15446807]
68. Datta K, Suman S, Kallakury BV, Fornace AJ Jr. Heavy ion radiation exposure triggered higher intestinal tumor frequency and greater beta-catenin activation than gamma radiation in APC(Min/+) mice. PLoS ONE. 2013; 8:e59295. [PubMed: 23555653]
69. Hei TK, Zhao Y, Zhou H, Ivanov V. Mechanism of radiation carcinogenesis: role of the TGFBI gene and the inflammatory signaling cascade. Adv Exp Med Biol. 2011; 720:163–170. [PubMed: 21901626]
70. Barcellos-Hoff MH, Lyden D, Wang TC. The evolution of the cancer niche during multistage carcinogenesis. Nat Rev Cancer. 2013; 13:511–518. [PubMed: 23760023]
71. Hall EJ, Brenner DJ. Cancer risks from diagnostic radiology: the impact of new epidemiological data. Br J Radiol. 2012; 85:e1316–e1317. [PubMed: 23175496]
72. Hlatky L, Hahnfeldt P. Beyond the cancer cell: progression-level determinants highlight the multiscale nature of carcinogenesis risk. Cancer Res. 2014 **(in press)**.
73. Kennedy AR, Davis JG, Carlton W, Ware JH. Effects of dietary antioxidant supplementation on the development of malignancies and other neoplastic lesions in mice exposed to proton or iron ion radiation. Radiation Research. 2008; 169:615–625. [PubMed: 18494549]
74. Ding LH, Park S, Peyton M, Girard L, Xie Y, Minna JD, Story MD. Distinct transcriptome profiles identified in normal human bronchial epithelial cells after exposure to gamma-rays and different elemental particles of high Z and energy. BMC Genomics. 2013; 14:372. [PubMed: 23724988]
75. Datta K, Suman S, Kallakury BV, Fornace AJ Jr. Exposure to heavy ion radiation induces persistent oxidative stress in mouse intestine. PLoS ONE. 2012; 7:e42224. [PubMed: 22936983]
76. Burns FJ, Zhao P, Xu G, Roy N, Loomis C. Fibroma induction in rat skin following single or multiple doses of 1.0 GeV/nucleus ^{56}Fe ions from the Brookhaven Alternating Gradient Synchrotron (AGS). Physica Medica. 2001; 17(Suppl 1):194–195. [PubMed: 11776259]
77. Burns FJ, Tang M-S, Frenkel K, Nadas A, Wu F, Uddin A, Zhang R. Induction and prevention of carcinogenesis in rat skin exposed to space radiation. Radiation and Environmental Biophysics. 2007; 46:195–199. [PubMed: 17387500]
78. Zhang R, Burns FJ, Chen H, Chen S, Wu F. Alterations in gene expression in rat skin exposed to 56Fe ions and dietary vitamin A acetate. Radiat Res. 2006; 165:570–581. [PubMed: 16669712]
79. Rabin BM, Joseph JA, Shukitt-Hale B. Effects of age and diet on the heavy particle-induced disruption of operant responding produced by a ground-based model for exposure to cosmic rays. Brain Research. 2005; 1036:122–129. [PubMed: 15725409]
80. Rabin BM, Shukitt-Hale B, Joseph J, Todd P. Diet as a factor in behavioral radiation protection following exposure to heavy particles. Gravitational & Space Biology Bulletin. 2005; 18:71–77. [PubMed: 16038094]
81. Das B, Bennett PV, Cutter NC, Sutherland JC, Sutherland BM. Melatonin protects human cells from clustered DNA damages, killing and acquisition of soft agar growth induced by X-rays or 970 MeV/n Fe ions. Int J Radiat Biol. 2011; 87:545–555. [PubMed: 21401316]
82. Eskiocak U, Kim SB, Roig AI, Kitten E, Batten K, Cornelius C, Zou YS, Wright WE, Shay JW. CDDO-Me protects against space radiation-induced transformation of human colon epithelial cells. Radiation Research. 2010; 174:27–36. [PubMed: 20681796]
83. Travis L, Ng A, Allan J, Pui C-H, Kennedy AR, Xu X, Purdy J, Applegate K, Yahalom J, et al. Second malignant neoplasms and cardiovascular disease following radiotherapy. Journal of the National Cancer Institute. 2012; 104:357–370. [PubMed: 22312134]
84. Travis LB, Ng AK, Allan JM, Pui CH, Kennedy AR, Xu XG, Purdy JA, Applegate K, Yahalom J, et al. Second malignant neoplasms and cardiovascular disease following radiotherapy. Health Phys. 2014; 106:229–246. [PubMed: 24378498]
85. NCRP, National Council on Radiation Protection and Measurements (NCRP). Second primary cancers and cardiovascular disease after radiation therapy. NCRP; 2011. Report No. 170

86. Yu T, Parks BW, Yu S, Srivastava R, Gupta K, Wu X, Khaled S, Chang PY, Kabarowski JH, Kucik DF. Iron-ion radiation accelerates atherosclerosis in apolipoprotein E-deficient mice. Radiation Research. 2011; 175:766–773. [PubMed: 21466380]

87. Soucy KG, Lim HK, Kim JH, Oh Y, Attarzadeh DO, Sevinc B, Kuo MM, Shoukas AA, Vazquez ME, Berkowitz DE. HZE ^{56}Fe-ion irradiation induces endothelial dysfunction in rat aorta: role of xanthine oxidase. Radiation Research. 2011; 176:474–485. [PubMed: 21787183]

88. Grabham P, Hu B, Sharma P, Geard C. Effects of ionizing radiation on three-dimensional human vessel models: differential effects according to radiation quality and cellular development. Radiat Res. 2011; 175:21–28. [PubMed: 21175343]

89. Grabham P, Bigelow A, Geard C. DNA damage foci formation and decline in two-dimensional monolayers and in three-dimensional human vessel models: differential effects according to radiation quality. Int J Radiat Biol. 2012; 88:493–500. [PubMed: 22449005]

90. Grabham P, Sharma P. The effects of radiation on angiogenesis. Vasc Cell. 2013; 5:19. [PubMed: 24160185]

91. Grabham P, Sharma P, Bigelow A, Geard C. Two distinct types of the inhibition of vasculogenesis by different species of charged particles. Vasc Cell. 2013; 5:16. [PubMed: 24044765]

92. Blakely EA, Kleiman NJ, Neriishi K, Chodick G, Chylack LT, Cucinotta FA, Minamoto A, Nakashima E, Kumagami T, et al. Radiation cataractogenesis: epidemiology and biology. Radiation Research. 2010; 173:709–717. [PubMed: 20426671]

93. Dynlacht JR. The role of age, sex and steroid sex hormones in radiation cataractogenesis. Radiat Res. 2013; 180:559–566. [PubMed: 24261552]

94. Kleiman NJ. Radiation cataract. Ann ICRP. 2012; 41:80–97. [PubMed: 23089007]

95. Rehani MM, Vano E, Ciraj-Bjelac O, Kleiman NJ. Radiation and cataract. Radiat Prot Dosimetry. 2011; 147:300–304. [PubMed: 21764807]

96. Little MP. A review of non-cancer effects, especially circulatory and ocular diseases. Radiat Environ Biophys. 2013; 52:435–449. [PubMed: 23903347]

97. Ainsbury EA, Bouffler SD, Dorr W, Graw J, Muirhead CR, Edwards AA, Cooper J. Radiation cataractogenesis: a review of recent studies. Radiat Res. 2009; 172:1–9. [PubMed: 19580502]

98. Dynlacht JR, Valluri S, Garrett J, Mendonca MS, Lopez JT, Caperell-Grant A, Bigsby RM. Age and hormonal status as determinants of cataractogenesis induced by ionizing radiation. I. Densely ionizing (high-LET) radiation. Radiat Res. 2011; 175:37–43. [PubMed: 21175345]

99. Chang PY, Bjornstad KA, Rosen CJ, McNamara MP, Mancini R, Goldstein LE, Chylack LT, Blakely EA. Effects of iron ions, protons and X rays on human lens cell differentiation. Radiat Res. 2005; 164:531–539. [PubMed: 16187763]

100. Chang PY, Bjornstad KA, Rosen CJ, Lin S, Blakely EA. Particle radiation alters expression of matrix metalloproteases resulting in ECM remodeling in human lens cells. Radiat Environ Biophys. 2007; 46:187–194. [PubMed: 17256179]

101. Blakely EA, Chang PY. Late effects from hadron therapy. Radiother Oncol. 2004; 73(Suppl 2):S134–S140. [PubMed: 15971329]

102. Henderson MA, Valluri S, Garrett J, Lopez JT, Caperell-Grant A, Mendonca MS, Rusek A, Bigsby RM, Dynlacht JR. Effects of estrogen and gender on cataractogenesis induced by high-LET radiation. Radiat Res. 2010; 173:191–196. [PubMed: 20095851]

103. Cucinotta FA, Manuel FK, Jones J, Iszard G, Murrey J, Djojonegro B, Wear M. Space radiation and cataracts in astronauts. Radiation Research. 2001; 156:460–466. [PubMed: 11604058]

104. Rastegar N, Eckart P, Mertz M. Radiation-induced cataract in astronauts and cosmonauts. Graefes Archive for Clinical & Experimental Ophthalmology. 2002; 240:543–547.

105. Dainiak N, Waselenko JK, Armitage JO, MacVittie TJ, Farese AM. The hematologist and radiation casulaties. Hematology. 2003:473–496. [PubMed: 14633795]

106. Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, Tsu H, Confer DL, Coleman CN, et al. Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. Annals of Internal Medicine. 2004; 140:1037–1051. [PubMed: 15197022]

107. Harding RK. Prodromal effects of radiation: pathways, models, and protection by antiemetics. Pharmacology & Therapeutics. 1988; 39:335–345. [PubMed: 3059374]

108. Hawthorn J, Cunningham D. Dexamethasone can potentiate the anti-emetic action of a 5HT3 receptor antagonist on cyclophosphamide induced vomiting in the ferret. British Journal of Cancer. 1990; 61:56–60. [PubMed: 2137008]
109. Lofters WS, Pater JL, Zee B, Dempsey E, Walde D, Moquin JP, Wilson K, Hoskins P, Guevin RM, et al. Phase III double-blind comparison of dolasetron mesylate and ondansetron and an evaluation of the additive role of dexamethasone in the prevention of acute and delayed nausea and vomiting due to moderately emetogenic chemotherapy. Journal of Clinical Oncology. 1997; 15:2966–2973. [PubMed: 9256141]
110. Priestman TJ. Clinical studies with ondansetron in the control of radiation-induced emesis. European Journal of Cancer & Clinical Oncology. 1989; 25:S29–S33. [PubMed: 2533896]
111. Nuth M, Kennedy AR. Mitigating effects of L-selenomethionine on low-dose iron ion radiation-induced changes in gene expression associated with cellular stress. Oncol Lett. 2013; 6:35–42. [PubMed: 23946774]
112. Sasse AD, Clark LG, Sasse EC, Clark OA. Amifostine reduces side effects and improves complete response rate during radiotherapy: results of a meta-analysis. International Journal of Radiation Oncology, Biology, Physics. 2006; 64:784–791.
113. Prieto Gonzalez EA, Fuchs AG, Sanchez GS. Amifostine (WR2721) confers DNA protection to in vivo cisplatin-treated murine peripheral blood leukocytes. Dose-Response. 2009; 7:234–246. [PubMed: 19809542]
114. Muller L, Moorervandelft C, Treskes M, Vermorken J, Vandervijgh W, Boer H. Properties of wr2721 (ethiofos) as modulator of Cisplatin-induced neurotoxicity studied at the ultrastructural level in the pond snail lymnaea-stagnalis. International Journal of Oncology. 1993; 2:701–710. [PubMed: 21573614]
115. Mukherjee N, Carroll BL, Spees JL, Delay ER. Pre-treatment with amifostine protects against cyclophosphamide-induced disruption of taste in mice. PLoS ONE. 2013; 8:e61607. [PubMed: 23626702]
116. Chen C, Tian L, Zhang M, Sun Q, Zhang X, Li X, Cao X, Liu Q, Li X, Hao L. Protective effect of amifostine on high-dose methotrexate-induced small intestinal mucositis in mice. Digestive Diseases & Sciences. 2013; 58:3134–3143. [PubMed: 23979434]
117. Carnes BA, Grdina DJ. In vivo protection by the aminothiol WR-2721 against neutron-induced carcinogenesis. International Journal of Radiation Biology. 1992; 61:567–576. [PubMed: 1349621]
118. Soref CM, Hacker TA, Fahl WE. A new orally active, aminothiol radioprotector-free of nausea and hypotension side effects at its highest radioprotective doses. International Journal of Radiation Oncology, Biology, Physics. 2012; 82:e701–e707.
119. Rose PG. Amifostine cytoprotection with chemotherapy for advanced ovarian carcinoma. Seminars in Oncology. 1996; 23:S83–S89.
120. Kligerman MM, Turrisi AT 3rd, Urtasun RC, Norfleet AL, Phillips TL, Barkley T, Rubin P. Final report on phase I trial of WR-2721 before protracted fractionated radiation therapy. International Journal of Radiation Oncology, Biology, Physics. 1988; 14:1119–1122.
121. Florczyk AP, Schurig JE, Bradner WT. Cisplatin-induced emesis in the ferret: a new animal model. Cancer Treatment Reports. 1982; 66:187–189. [PubMed: 7198011]
122. King GL. Characterization of radiation-induced emesis in the ferret. Radiation Research. 1988; 114:599–612. [PubMed: 3375446]
123. Hamm PC, Bakker EJ, van den Berg AP, van den Aardweg GJ, Visser AG, Levendag PC. Single dose irradiation response of pig skin: a comparison of brachytherapy using a single, high dose rate iridium-192 stepping source with 200 kV X-rays. Br J Radiol. 2000; 73:762–770. [PubMed: 11089469]
124. Zacharias T, Dörr W, Enghardt W, Haberer T, Krämer M, Kumpf R, Röthig H, Scholz M, Weber U, et al. Acute response of pig skin to irradiation with 12C-ions or 200 kV X-rays. Acta Oncologica. 1997; 36:637–642. [PubMed: 9408156]
125. Hopewell JW, Robbins ME, van den Aardweg GJ, Morris GM, Ross GA, Whitehouse E, Horrobin DF, Scott CA. The modulation of radiation-induced damage to pig skin by essential fatty acids. Br J Cancer. 1993; 68:1–7. [PubMed: 8391301]

126. Archambeau JO, Fairchild RG, Brenneis HJ. The response of the skin of swine to increasing absorbed doses of radiation from a thermal neutron beam, a degraded fission neutron beam, and the 10B(n, alpha)7Li reaction. Radiation Research. 1971; 45:145–165. [PubMed: 5539702]

127. Yamamoto K, Matsunaga S, Matsui M, Takeda N, Yamatodani A. Pica in mice as a new model for the study of emesis. Methods & Findings in Experimental & Clinical Pharmacology. 2002; 24:135–138. [PubMed: 12087874]

128. Louisy F, Guezennec CY, Guell A. Leg vein hemodynamics during bedrests simulating lunar trip. Journal of Gravitational Physiology. 1994; 1:P100–P101. [PubMed: 11538729]

129. Cengel KA, Diffenderfer ES, Avery S, Kennedy AR, McDonough J. Using electron beam radiation to simulate the dose distribution for whole body solar particle event proton exposure. Radiation & Environmental Biophysics. 2010; 49:715–721. [PubMed: 20725839]

130. Wilson JM, Sanzari JK, Diffenderfer ES, Yee SS, Seykora JT, Maks C, Ware JH, Litt HI, Reetz JA, et al. Acute biological effects of simulating the whole-body radiation dose distribution from a solar particle event using a porcine model. Radiation Research. 2011; 176:649–659. [PubMed: 21859326]

131. Diffenderfer ES, Dolney D, Schaettler M, Sanzari JK, McDonough J, Cengel KA. Monte Carlo modeling in CT-based geometries: dosimetry for biological modeling experiments with particle beam radiation. Journal of Radiation Research. 2013 **(in press)** Dec 2015 (Epub ahead of print) PMID: 24309720.

132. Wambi C, Sanzari J, Nuth M, Davis J, Ko Y-H, Sayers CM, Baran M, Ware JH, Kennedy AR. Dietary antioxidants protect hematopoietic cells and improve animal survival following total-body irradiation. Radiation Research. 2008; 169:384–396. [PubMed: 18363433]

133. Maks CJ, Wan XS, Ware JH, Romero-Weaver AL, Sanzari JK, Wilson JM, Rightnar S, Wroe AJ, Koss P, et al. Analysis of white blood cell counts in mice following gamma or proton radiation exposure. Radiation Research. 2011; 176:170–176. [PubMed: 21476859]

134. Romero-Weaver AL, Diffenderfer ES, Lin L, Kennedy AR. Effects of solar particle event-like proton radiation and/or simulated microgravity on circulating mouse blood cells. Gravitational and Space Biology. 2013 **(in press)**.

135. Romero-Weaver AL, Wan XS, Diffenderfer ES, Lin L, Kennedy AR. Kinetics of neutrophils in mice exposed to radiation and/or granulocyte colony-stimulating factor treatment. Radiation Research. 2013; 180:177–188. [PubMed: 23829559]

136. Sanzari JK, Wilson JM, Wagner EB, Kennedy AR. The combined effects of reduced weightbearing and ionizing radiation on splenic lymphocyte population and function. International Journal of Radiation Biology. 2011; 87:1033–1038. [PubMed: 21770700]

137. Ware JH, Sanzari J, Avery S, Sayers C, Krigsfeld G, Nuth M, Wan XS, Rusek A, Kennedy AR. Effects of proton radiation dose, dose rate and dose fractionation on hematopoietic cells in mice. Radiation Research. 2010; 174:325–330. [PubMed: 20726731]

138. Sanzari JK, Romero-Weaver AL, James G, Krigsfeld G, Lin L, Diffenderfer ES, Kennedy AR. Leukocyte activity is altered in a ground based murine model of microgravity and proton radiation exposure. PLoS One. 2013; 8:e71757. [PubMed: 23977138]

139. Wambi CO, Sanzari JK, Sayers CM, Nuth M, Zhou Z, Davis J, Finnberg N, Lewis-Wambi JS, Ware JH, et al. Protective effects of dietary antioxidants on proton total-body irradiation-mediated hematopoietic cell and animal survival. Radiation Research. 2009; 172:175–186. [PubMed: 19630522]

140. Sanzari JK, Wan XS, Rusek A, Diffenderfer ES, Kennedy AR. Acute hematological effects in mice exposed to the expected doses, dose-rates, and energies of solar particle event-like proton radiation. Life Sciences in Space Research. 2014 **(in press)**.

141. Sanzari JK, Wan XS, Krigsfeld GS, Wroe AJ, Gridley DS, Kennedy AR. The effects of gamma and proton radiation exposure on hematopoietic cell counts in the ferret model. Gravitational and Space Biology. 2013; 1:79–94.

142. Krigsfeld GS, Savage AR, Billings PC, Lin L, Kennedy AR. Evidence for radiation induced disseminated intravascular coagulation as a major cause of radiation induced death in ferrets. International Journal of Radiation Oncology, Biology, Physics. 2013 **(in press)**.

143. Sanzari JK, Wan XS, Wroe AJ, Rightnar S, Cengel KA, Diffenderfer ES, Krigsfeld GS, Gridley DS, Kennedy AR. Acute hematological effects of solar particle event proton radiation in the porcine model. Radiation Research. 2013; 180:7–16. [PubMed: 23672458]

144. Sanzari JK, Wan XS, Diffenderfer ES, Cengel KA, Kennedy AR. Relative biological effectiveness of simulated solar particle event proton radiation to induce acute hematological change in the porcine model. Journal of Radiation Research. 2013; 2013 2013 Sep 2011 (Epub ahead of print) PMID: 24027300.

145. Romero-Weaver AL, Wan XS, Diffenderfer ES, Lin L, Kennedy AR. Effect of SPE-like proton or photon radiation on the kinetics of mouse peripheral blood cells and radiation biological effectiveness determinations. Astrobiology. 2013; 13:570–577. [PubMed: 23980767]

146. Romero-Weaver AL, Kennedy AR. Comparison of two methods for the determination of the effects of ionizing radiation on blood cell counts in mice. International Journal of Biomedical Science. 2012; 8:7–15. [PubMed: 23450807]

147. Billings PC, Romero-Weaver AL, Kennedy AR. Effect of gender on the radiation sensitivity of murine blood cells. Gravitational and Space Biology. 2013 **(in press)**.

148. Wilson JM, Krigsfeld GS, Sanzari JK, Wagner EB, Mick R, Kennedy AR. Comparison of hindlimb unloading and partial weight suspension models for spaceflight-type condition induced effects on white blood cells. Advances in Space Research. 2012; 49:237–248. [PubMed: 23766550]

149. Wagner EB, Granzella NP, Saito H, Newman DJ, Young LR, Bouxsein ML. Partial weight suspension: A novel murine model for investigating adaptation to reduced musculoskeletal loading. Journal of Applied Physiology. 2010; 109:350–357. [PubMed: 20522735]

150. Gridley DS, Freeman TL, Makinde AY, Wroe AJ, Luo-Owen X, Tian J, Mao XW, Rightnar S, Kennedy AR, et al. Comparison of proton and electron radiation effects on biological responses in liver, spleen and blood. International Journal of Radiation Biology. 2011; 87:1173–1181. [PubMed: 22035456]

151. Li M, Holmes V, Ni H, Sanzari JK, Kennedy AR, Weissman D. Hindlimb suspension and SPE-like radiation impairs clearance of bacterial infections. PLoS One. 2014; 9:e85665. [PubMed: 24454913]

152. Zhou Y, Ni H, Li M, Sanzari JK, Diffenderfer ES, Lin L, Kennedy AR, Weissman D. Effect of solar particle event radiation and hindlimb suspension on gastrointestinal tract bacterial translocation and immune activation. PLoS ONE. 2012; 7:e44329. [PubMed: 23028522]

153. Ni H, Balint K, Zhou Y, Gridley DS, Maks C, Kennedy AR, Weissman D. Effect of solar particle event radiation on gastrointestinal tract bacterial translocation and immune activation. Radiation Research. 2011; 175:485–492. [PubMed: 21294608]

154. Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, Landay A, Martin J, Sinclair E, et al. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. Journal of Infectious Diseases. 2009; 199:1177–1185. [PubMed: 19265479]

155. Townsend LW. Implications of the space radiation environment for human exploration in deep space. Radiation Protection Dosimetry. 2005; 115:44–50. [PubMed: 16381680]

156. Brenchley JM, Paiardini M, Knox KS, Asher AI, Cervasi B, Asher TE, Scheinberg P, Price DA, Hage CA, et al. Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. Blood. 2008; 112:2826–2835. [PubMed: 18664624]

157. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nature Medicine. 2006; 12:1365–1371.

158. Crucian B, Sams C. Immune system dysregulation during spaceflight: clinical risk for exploration-class missions. Journal of Leukocyte Biology. 2009; 86:1017–1018. [PubMed: 19875627]

159. Gueguinou N, Huin-Schohn C, Bascove M, Bueb JL, Tschirhart E, Legrand-Frossi C, Frippiat JP. Could spaceflight-associated immune system weakening preclude the expansion of human presence beyond Earth's orbit? Journal of Leukocyte Biology. 2009; 86:1027–1038. [PubMed: 19690292]

160. Baqai FP, Gridley DS, Slater JM, Luo-Owen X, Stodieck LS, Ferguson V, Chapes SK, Pecaut MJ. Effects of spaceflight on innate immune function and antioxidant gene expression. Journal of Applied Physiology. 2009; 106:1935–1942. [PubMed: 19342437]

161. Srinivasan A, McSorley SJ. Pivotal advance: exposure to LPS suppresses CD4+ T cell cytokine production in Salmonella-infected mice and exacerbates murine typhoid. Journal of Leukocyte Biology. 2007; 81:403–411. [PubMed: 16916961]

162. Bukh AR, Melchjorsen J, Offersen R, Jensen JM, Toft L, Stovring H, Ostergaard L, Tolstrup M, Sogaard OS. Endotoxemia is associated with altered innate and adaptive immune responses in untreated HIV-1 infected individuals. PLoS ONE. 2011; 6:e21275. [PubMed: 21731690]

163. Lee PI, Ciccone EJ, Read SW, Asher A, Pitts R, Douek DC, Brenchley JM, Sereti I. Evidence for translocation of microbial products in patients with idiopathic CD4 lymphocytopenia. Journal of Infectious Diseases. 2009; 199:1664–1670. [PubMed: 19432548]

164. Jagannathan M, Hasturk H, Liang Y, Shin H, Hetzel JT, Kantarci A, Rubin D, McDonnell ME, Van Dyke TE, et al. TLR cross-talk specifically regulates cytokine production by B cells from chronic inflammatory disease patients. Journal of Immunology. 2009; 183:7461–7470.

165. Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R. Toll-like receptors control activation of adaptive immune responses. Nature Immunology. 2001; 2:947–950. [PubMed: 11547333]

166. Medvedev AE, Sabroe I, Hasday JD, Vogel SN. Tolerance to microbial TLR ligands: molecular mechanisms and relevance to disease. Journal of Endotoxin Research. 2006; 12:133–150. [PubMed: 16719986]

167. Kaur I, Simons ER, Kapadia AS, Ott CM, Pierson DL. Effect of spaceflight on ability of monocytes to respond to endotoxins of gram-negative bacteria. Clinical & Vaccine Immunology: CVI. 2008; 15:1523–1528. [PubMed: 18768671]

168. Holub M, Lawrence DA, Mondal TK. Effects of murine endotoxemia on lymphocyte subsets and clearance of staphylococcal pulmonary infection. Folia Microbiologica. 2006; 51:469–472. [PubMed: 17176769]

169. Kariko K, Weissman D, Welsh FA. Inhibition of toll-like receptor and cytokine signaling--a unifying theme in ischemic tolerance. Journal of Cerebral Blood Flow & Metabolism. 2004; 24:1288–1304. [PubMed: 15545925]

170. Elson CO, Cong Y, McCracken VJ, Dimmitt RA, Lorenz RG, Weaver CT. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunological Reviews. 2005; 206:260–276. [PubMed: 16048554]

171. Obermeier F, Dunger N, Strauch UG, Hofmann C, Bleich A, Grunwald N, Hedrich HJ, Aschenbrenner E, Schlegelberger B, et al. CpG motifs of bacterial DNA essentially contribute to the perpetuation of chronic intestinal inflammation. Gastroenterology. 2005; 129:913–927. [PubMed: 16143131]

172. Caradonna L, Mastronardi ML, Magrone T, Cozzolongo R, Cuppone R, Manghisi OG, Caccavo D, Pellegrino NM, Amoroso A, et al. Biological and clinical significance of endotoxemia in the course of hepatitis C virus infection. Current Pharmaceutical Design. 2002; 8:995–1005. [PubMed: 11945146]

173. Schafer C, Parlesak A, Schutt C, Bode JC, Bode C. Concentrations of lipopolysaccharide-binding protein, bactericidal/permeability-increasing protein, soluble CD14 and plasma lipids in relation to endotoxaemia in patients with alcoholic liver disease. Alcohol & Alcoholism. 2002; 37:81–86. [PubMed: 11825862]

174. Mao XW, Mekonnen T, Kennedy AR, Gridley DS. Differential expression of oxidative stress and extracellular matrix remodeling genes in low- or high-dose-rate photon-irradiated skin. Radiation Research. 2011; 176:187–197. [PubMed: 21574862]

175. Tomura M, Honda T, Tanizaki H, Otsuka A, Egawa G, Tokura Y, Waldmann H, Hori S, Cyster JG, et al. Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice. Journal of Clinical Investigation. 2010; 120:883–893. [PubMed: 20179354]

176. Riezu-Boj JI, Larrea E, Aldabe R, Guembe L, Casares N, Galeano E, Echeverria I, Sarobe P, Herrero I, et al. Hepatitis C virus induces the expression of CCL17 and CCL22 chemokines that attract regulatory T cells to the site of infection. Journal of Hepatology. 2011:422–431. [PubMed: 21129807]

177. Czarnik TR. Medical emergencies in space. 1988

178. Dietlein RS, Berry LF, Johnston CA. Biomedical results of Apollo. Scientific and Technical Information Office, National Aeronautics and Space. 1975

179. Taylor GR. Recovery of medically important microorganisms from Apollo astronauts. Aerosp. Med. 1974; 45:824–828. [PubMed: 4153037]

180. Taylor GR, Dardano JR. Human cellular immune responsiveness following space flight. Aviation Space & Environmental Medicine. 1983; 54(S55)

181. Taylor GR, Neale LS, Dardano JR. Immunological analyses of U.S. Space Shuttle crewmembers. Aviation Space & Environmental Medicine. 1986; 57:213–217.

182. Taylor GR, Konstantinova I, Sonnenfeld G, Jennings R. Changes in the immune system during and after spaceflight. Advances in Space Biology & Medicine. 1997; 6:1–32. [PubMed: 9048132]

183. Taylor GR, Zaloguev SN. Medically important micro-organisms recovered from Apollo-Soyuz Test Project (ASTP) crew members. Life Sci Space Res. 1977; 15:207–212. [PubMed: 11958217]

184. Fabre KM, Ramaiah L, Dregalla RC, Desaintes C, Weil MM, Bailey SM, Ullrich RL. Murine Prkdc polymorphisms impact DNA-PKcs function. Radiation Research. 2011; 175:493–500. [PubMed: 21265624]

185. Mori N, Matsumoto Y, Okumoto M, Suzuki N, Yamate J. Variations in Prkdc encoding the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and susceptibility to radiation-induced apoptosis and lymphomagenesis. Oncogene. 2001; 20:3609–3619. [PubMed: 11439324]

186. Okayasu R, Suetomi K, Yu Y, Silver A, Bedford JS, Cox R, Ullrich RL. A deficiency in DNA repair and DNA-PKcs expression in the radiosensitive BALB/c mouse. Cancer Research. 2000; 60:4342–4345. [PubMed: 10969773]

187. Pierson, DL.; McGinnis, MR.; Viktorov, AN. Microbiological Contamination. In: Nicogossian, AE.; Mohler, SR.; Gazenko, OG.; Grigoryev, AI., editors. Space Biology and Medicine. Washington, D. C.: American Institute of Aeronautics and Astronautics, Inc.; 1994. p. 77-93.

188. Nickerson CA, Ott CM, Wilson JW, Ramamurthy R, Pierson DL. Microbial responses to microgravity and other low-shear environments. Microbiol Mol Biol Rev. 2004; 68:345–361. [PubMed: 15187188]

189. Feyer PC, Stewart AL, Titlbach OJ. Aetiology and prevention of emesis induced by radiotherapy. Supportive Care in Cancer. 1998; 6:253–260. [PubMed: 9629879]

190. T. I. G. f. A. R. i. Radiotherapy, Radiation-induced emesis: a prospective observational multicenter Italian trial. The Italian Group for Antiemetic Research in Radiotherapy. International Journal of Radiation Oncology, Biology, Physics. 1999; 44:619–625.

191. King GL. Animal models in the study of vomiting. Canadian Journal of Physiology & Pharmacology. 1990; 68:260–268. [PubMed: 2178751]

192. Andrews PL, Davis CJ, Bingham S, Davidson HI, Hawthorn J, Maskell L. The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity. Canadian Journal of Physiology & Pharmacology. 1990; 68:325–345. [PubMed: 2178756]

193. McClellan, GE.; Anno, GH.; King, GL.; Young, RW. Evaluation of a predictive algorithm for the human emetic response to protracted irradiation using ferret data. In: Bianchi, AL.; Grelot, L.; Miller, AD.; King, GL., editors. Mechanisms and Control of Emesis. Colloque INSERM/John Libbey Eurotext Ltd; 1992.

194. Sanzari JK, Wan XS, Krigsfeld GS, King GL, Miller A, Mick R, Gridley DS, Wroe AJ, Rightnar S, et al. Effects of solar particle event proton radiation on parameters related to ferret emesis. Radiation Research. 2013; 180:166–176. [PubMed: 23883319]

195. Rabin BM, Hunt WA, Wilson ME, Joseph JA. Emesis in ferrets following exposure to different types of radiation: a dose-response study. Aviation Space & Environmental Medicine. 1992; 63:702–705.

196. King GL, Rabin BM, Weatherspoon JK. 5-HT3 receptor antagonists ameliorate emesis in the ferret evoked by neutron or proton radiation. Aviation Space & Environmental Medicine. 1999; 70:485–492.

197. Rabin BM, Joseph JA, Hunt WA, Dalton TB, Kandasamy SB, Harris AH, Ludewigt B. Behavioral endpoints for radiation injury. Advances in Space Research. 1994; 14:457–466. [PubMed: 11539983]

198. Hoffman M, Monroe DM 3rd. A cell-based model of hemostasis. Thrombosis & Haemostasis. 2001; 85:958–965. [PubMed: 11434702]

199. Furie B, Furie BC. The molecular basis of blood coagulation. Cell. 1988; 53:505–518. [PubMed: 3286010]

200. Davie EW, Fujikawa K, Kisiel W. The coagulation cascade: initiation, maintenance, and regulation. Biochemistry. 1991; 30:10363–10370. [PubMed: 1931959]

201. Sporn LA, Rubin P, Marder VJ, Wagner DD. Irradiation induces release of von Willebrand protein from endothelial cells in culture. Blood. 1984; 64:567–570. [PubMed: 6611184]

202. Jahroudi N, Ardekani AM, Greenberger JS. Ionizing irradiation increases transcription of the von Willebrand factor gene in endothelial cells. Blood. 1996; 88:3801–3814. [PubMed: 8916944]

203. Goldin-Lang P, Niebergall F, Antoniak S, Szotowski B, Rosenthal P, Pels K, Schultheiss HP, Rauch U. Ionizing radiation induces upregulation of cellular procoagulability and tissue factor expression in human peripheral blood mononuclear cells. Thrombosis Research. 2007; 120:857–864. [PubMed: 17337288]

204. Weisbach V, Strobel J, Hahn B, Rodel F, Lotter M, Zingsem J, Ringwald J, Eckstein R. Effect of gamma irradiation with 30 Gy on the coagulation system in leukoreduced fresh-frozen plasma. Transfusion. 2007; 47:1658–1665. [PubMed: 17725731]

205. Krigsfeld GS, Sanzari JK, Kennedy AR. The effects of proton radiation on the prothrombin and partial thromboplastin times of irradiated ferrets. International Journal of Radiation Biology. 2012; 88:327–334. [PubMed: 22221163]

206. Yuan S, Ferrell C, Chandler WL. Comparing the prothrombin time INR versus the APTT to evaluate the coagulopathy of acute trauma. Thrombosis Research. 2007; 120:29–37. [PubMed: 16887171]

207. Ng VL. Liver disease, coagulation testing, and hemostasis. Clinics in Laboratory Medicine. 2009; 29:265–282. [PubMed: 19665678]

208. Krigsfeld GS, Savage AR, Sanzari JK, Wroe AJ, Gridley DS, Kennedy AR. Mechanism of hypocoagulability in proton-irradiated ferrets. International Journal of Radiation Biology. 2013; 89:823–831. [PubMed: 23651328]

209. Hay KL, Bull BS. Rapid-SF: a rapid whole-blood screen for soluble fibrin monomer. Thrombosis & Haemostasis. 2002; 88:773–780. [PubMed: 12428093]

210. Dorr H, Meineke V. Acute radiation syndrome caused by accidental radiation exposure - therapeutic principles. BMC Medicine. 2011; 9:126. [PubMed: 22114866]

211. York JM, Blevins NA, Meling DD, Peterlin MB, Gridley DS, Cengel KA, Freund GG. The biobehavioral and neuroimmune impact of low-dose ionizing radiation. Brain, Behavior, & Immunity. 2012; 26:218–227.

212. York JM, McDaniel AW, Blevins NA, Guillet RR, Allison SO, Cengel KA, Freund GG. Individually ventilated cages cause chronic low-grade hypoxia impacting mice hematologically and behaviorally. Brain, Behavior, & Immunity. 2012; 26:951–958.

213. York, JM. Ideals. Urbana, Illinois: University of Illinois; 2012. Behavioral changes in response to low-grade non-infectious stimuli: findings and potential neuroimmune mechanisms (2012-05-22).

214. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. Current Vascular Pharmacology. 2012; 10:4–18. [PubMed: 22112350]

215. Sung CP, Arleth AJ, Shikano K, Berkowitz BA. Characterization and function of bradykinin receptors in vascular endothelial cells. Journal of Pharmacology & Experimental Therapeutics. 1988; 247:8–13. [PubMed: 2902216]

216. Soucy KG, Lim HK, Benjo A, Santhanam L, Ryoo S, Shoukas AA, Vazquez ME, Berkowitz DE. Single exposure gamma-irradiation amplifies xanthine oxidase activity and induces endothelial

dysfunction in rat aorta. Radiation & Environmental Biophysics. 2007; 46:179–186. [PubMed: 17256177]

217. Soucy KG, Lim HK, Attarzadeh DO, Santhanam L, Kim JH, Bhunia AK, Sevinc B, Ryoo S, Vazquez ME, et al. Dietary inhibition of xanthine oxidase attenuates radiation-induced endothelial dysfunction in rat aorta. Journal of Applied Physiology. 2010; 108:1250–1258. [PubMed: 20167676]
218. Balter S, Hopewell JW, Miller DL, Wagner LK, Zelefsky MJ. Fluoroscopically guided interventional procedures: a review of radiation effects on patients' skin and hair. Radiology. 2010; 254:326–341. [PubMed: 20093507]
219. NCRP, National Council on Radiation Protection and Measurements (NCRP). Report No. 168. Radiation dose management for fluoroscopically-guided interventional medical procedures. NCRP. 2010
220. Mader TH, Gibson CR, Pass AF, Kramer LA, Lee AG, Fogarty J, Tarver WJ, Dervay JP, Hamilton DR, et al. Optic disc edema, globe flattening, choroidal folds, and hyperopic shifts observed in astronauts after long-duration space flight. Ophthalmology. 2011; 118:2058–2069. [PubMed: 21849212]
221. Sanzari JK, Muehlmatt A, Savage A, Lin L, Kennedy AR. Increased intracranial pressure in mini-pigs exposed to simulated solar particle event radiation. Acta Astronautica. 2014; 94:807–812. (published online: 2013 Nov 2019).
222. Datta K, Suman S, Trani D, Doiron K, Rotolo JA, Kallakury BV, Kolesnick R, Cole MF, Fornace AJ Jr. Accelerated hematopoietic toxicity by high energy $^{(56)}$Fe radiation. Int J Radiat Biol. 2012; 88:213–222. [PubMed: 22077279]
223. Suman S, Datta K, Trani D, Laiakis EC, Strawn SJ, Fornace AJ Jr. Relative biological effectiveness of ^{12}C and ^{28}Si radiation in C57BL/6J mice. Radiat Environ Biophys. 2012; 51:303–309. [PubMed: 22562428]
224. Krigsfeld GS, Kennedy AR. Is disseminated intravascular coagulation the major cause of mortality from radiation at relatively low whole body doses? Radiation Research. 2013; 180:231–234. [PubMed: 23944605]
225. Kennedy AR, Ware JH, Carlton W, Davis JG. Suppression of the later stages of radiation induced carcinogenesis by antioxidant dietary formulations. Radiation Research. 2011; 176:62–70. [PubMed: 21520997]
226. Davis JG, Wan XS, Ware JH, Kennedy AR. Dietary supplements reduce the cataractogenic potential of proton and HZE-particle radiation in mice. Radiation Research. 2010; 173:353–361. [PubMed: 20199220]
227. Sanzari JK, Wambi C, Lewis-Wambi JS, Kennedy AR. Antioxidant dietary supplementation in mice exposed to proton radiation attenuates expression of programmed cell death-associated genes. Radiation Research. 2011; 175:650–656. [PubMed: 21443425]
228. Finnberg N, Wambi C, Ware JH, Kennedy AR, El-Deiry WS. Gamma-radiation (GR) triggers a unique gene expression profile associated with cell death compared to proton radiation (PR) in mice in vivo. Cancer Biology & Therapy. 2008; 7:2023–2033. [PubMed: 19106632]
229. Hall, EJ. Linear energy transfer and relative biological effectiveness. In: Hall, EJ., editor. Radiobiology for the Radiologist. Philadelphia, PA: Lippincott Williams & Wilkins; 2000. p. 112-123.
230. Tsuruoka C, Suzuki M, Kanai T, Fujitaka K. LET and ion species dependence for cell killing in normal human skin fibroblasts. Radiation Research. 2005; 163:494–500. [PubMed: 15850410]
231. Tsuruoka C, Suzuki M, Hande MP, Furusawa Y, Anzai K, Okayasu R. The difference in LET and ion species dependence for induction of initially measured and non-rejoined chromatin breaks in normal human fibroblasts. Radiation Research. 2008; 170:163–171. [PubMed: 18666815]
232. Hirayama R, Ito A, Tomita M, Tsukada T, Yatagai F, Noguchi M, Matsumoto Y, Kase Y, Ando K. Contributions of direct and indirect actions in cell killing by high-LET radiations. Radiation Research. 2009; 171:212–218. [PubMed: 19267547]
233. Finnberg N, Wambi C, Kennedy AR, El-Deiry WS. The effects of antioxidants on gene expression following gamma-radiation (GR) and proton radiation (PR) in mice in vivo. Cell Cycle. 2013; 12:2241–2247.

234. Whaley JT, Kirk M, Cengel K, McDonough J, Bekelman J, Christodouleas JP. Protective effect of transparent film dressing on proton therapy induced skin reactions. Radiation Oncology Investigations. 2013; 8:19.

235. Romero-Weaver AL, Ni J, Lin L, Kennedy AR. Orally administered fructose increases the numbers of peripheral lymphocytes reduced by exposure of mice to gamma or SPE-like proton radiation. Life Sciences in Space Research. 2013 **(in press)**.

236. Wan XS, Zhou Z, Kennedy AR. Adaptation of the dichlorofluorescein assay for detection of radiation induced oxidative stress in cultured cells. Radiation Research. 2003; 160:622–630. [PubMed: 14640785]

237. Wan XS, Zhou Z, Ware JH, Kennedy AR. Standardization of a fluorometric assay for measuring oxidative stress in irradiated cells. Radiation Research. 2005; 163:232–240. [PubMed: 15658900]

238. Wan XS, Bloch P, Ware JH, Zhou Z, Donahue JJ, Guan J, Stewart J, Kennedy AR. Detection of oxidative stress induced by low and high linear energy transfer radiation in cultured human epithelial cells. Radiation Research. 2005; 163:364–368. [PubMed: 15799690]

239. van Zandwijk N. N-acetylcysteine (NAC) and glutathione (GSH): antioxidant and chemopreventive properties, with special reference to lung cancer. J. Cell. Biochem. (Suppl.). 1995; 22:24–32. [PubMed: 8538205]

240. Bump EA, Brown JM. Role of glutathione in the radiation response of mammalian cells in vitro and in vivo. Pharmacol. Ther. 1990; 47:117–136. [PubMed: 2195553]

241. Das KC, Lewis-Molock Y, White CW. Activation of NF-kappa B and elevation of MnSOD gene expression by thiol reducing agents in lung adenocarcinoma (A549) cells. American Journal of Physiology - Lung Cellular & Molecular Physiology. 1995; 269:L588–L602.

242. Murley JS, Kataoka Y, Hallahan DE, Roberts JC, Grdina DJ. Activation of NFkappaB and MnSOD gene expression by free radical scavengers in human microvascular endothelial cells. Free Radical Biology & Medicine. 2001; 30:1426–1439. [PubMed: 11390188]

243. Murley JS, Kataoka Y, Cao D, Li JJ, Oberley LW, Grdina DJ. Delayed radioprotection by NFkappaB-mediated induction of Sod2 (MnSOD) in SA-NH tumor cells after exposure to clinically used thiol-containing drugs. Radiation Research. 2004; 162:536–546. [PubMed: 15624308]

244. Oberley LW, St. Clair DK, Autor AP, Oberley TD. Increase in manganese superoxide dismutase activity in the mouse heart after X-irradiation. Arch. Biochem. Biophy. 1987; 254:69–80.

245. St. Clair DK, Wan XS, Overley TD, Muse KE, St. Clair WH. Suppression of radiation-induced neoplastic transformation by overexpression of mitochondrial superoxide dismutase. Mol. Carcinogenesis. 1992; 6:238–242.

246. Guo G, Yan-Sanders Y, Lyn-Cook BD, Wang T, Tamae D, Ogi J, Khaletskiy A, Li Z, Weydert C, et al. Manganese superoxide dismutase-mediated gene expression in radiation-induced adaptive responses. Molecular & Cellular Biology. 2003; 23:2362–2378. [PubMed: 12640121]

247. Rose RC. Ascorbic acid metabolism in protection against free radicals: a radiation model. Biom. Biophy. Res. Comm. 1990; 169:430–460.

248. Wolf R, Wolf D, Ruocco V. Vitamin E: the radical protector. J. Eur. Acad. Dermatol. Venereol. 1998; 10:103–117. [PubMed: 9553906]

249. Jacob RA, Burri BJ. Oxidative damage and defense. Am. J. Clin. Nutr. 1996; 63:985S–990S. [PubMed: 8644698]

250. Packer L, Witt EH, Tritschler HJ. Alpha-Lipoic acid as a biological antioxidant. Free Rad. Biol. Med. 1995; 19:227–250. [PubMed: 7649494]

251. Reed LJ. Multienzyme complex. Acc. Chem. Res. 1974; 7:40–46.

252. Biewenga GP, Haenen GR, Bast A. The pharmacology of the antioxidant lipoic acid. General Pharmacol. 1997; 29:315–331.

253. Marangon K, Devaraj S, Tirosh O, Packer L, Jialal I. Comparison of the effect of alpha-lipoic acid and alpha-tocopherol supplementation on measures of oxidative stress. Free Rad. Biol. Med. 1999; 27:1114–1121. [PubMed: 10569644]

254. Ramakrishnan N, Wolfe WW, Catravas GN. Radioprotection of hematopoietic tissues in mice by lipoic acid. Radiation Research. 1992; 130:360–365. [PubMed: 1594763]

255. Bantseev V, Bhardwaj R, Rathbun W, Nagasawa H, Trevithick JR. Antioxidants and cataract: (cataract induction in space environment and application to terrestrial aging cataract). Biochem. Mol. Biol. Intl. 1997; 42:1189–1197.

256. Michiels C, Raes M, Toussaint O, Remacle J. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Rad. Biol. Med. 1994; 17:235–248. [PubMed: 7982629]

257. Mustacich D, Powis G. Thioredoxin reductase. Biochem. J. 2000; 346:1–8. [PubMed: 10657232]

258. Frost DV, Lish PM. Selenium in biology. Ann. Rev. Pharmacol. 1975; 15:259–284. [PubMed: 807152]

259. Kennedy, AR. Anticarcinogenic activity of protease inhibitors: Overview. In: Troll, W.; Kennedy, AR., editors. Protease inhibitors as cancer chemopreventive agents. New York: Plenum Press; 1993. p. 9-64.

260. Kennedy AR. Chemopreventive agents: protease inhibitors. Pharmacol. Ther. 1998; 78:167–209. [PubMed: 9690817]

261. Kennedy AR. The Bowman-Birk Inhibitor from soybeans as an anticarcinogenic agent. Am. J. Clin. Nutr. 1998; 68(suppl.):1406s–1412s. [PubMed: 9848508]

262. Kennedy, AR. The status of human trials utilizing Bowman-Birk inhibitor concentrate from soybeans. In: Sugano, M., editor. Soy in Health and Disease Prevention. Boca Raton, Florida: CRC Press, Taylor & Francis Group, LLC; 2005. p. 207-223.

263. Dittmann K, Loffler H, Bamberg M, Rodemann HP. Bowman-Birk proteinase inhibitor (BBI) modulates radiosensitivity and radiation-induced differentiation of human fibroblasts in culture. Radiother. Oncol. 1995; 34:137–143. [PubMed: 7597212]

264. Dittmann KH, Gueven N, Mayer C, Ohneseit P, Zell R, Begg AC, Rodemann HP. The presence of wild-type TP53 is necessary for the radioprotective effect of the Bowman-Birk proteinase inhibitor in normal fibroblasts. Radiation Research. 1998; 150:648–655. [PubMed: 9840184]

265. Dittmann KH, Gueven N, Mayer C, Rodemann HP. The radioprotective effect of BBI is associated with the activation of DNA repair-relevant genes. International Journal of Radiation Biology. 1998; 74:225–230. [PubMed: 9712551]

266. Dittmann KH, Dikomey E, Mayer C, Rodemann HP. The Bowman-Birk protease inhibitor enhances clonogenic cell survival of ionizing radiation-treated nucleotide excision repair-competent cells but not of xeroderma pigmentosum cells. International Journal of Radiation Biology. 2000; 76:223–229. [PubMed: 10716643]

267. Dittmann KH, Gueven N, Mayer C, Rodemann HP. Characterization of the amino acids essential for the photo- and radioprotective effects of a Bowman-Birk protease inhibitor-derived nonapeptide. Protein Engineering. 2001; 14:157–160. [PubMed: 11342711]

268. Dittmann KH, Mayer C, Rodemann HP. Radioprotection of normal tissue to improve radiotherapy: the effect of the Bowman Birk protease inhibitor. Current Medicinal Chemistry - Anti-Cancer Agents. 2003; 3:360–363. [PubMed: 12871082]

269. Kennedy CW, Donahue JJ, Wan XS. Effects of Bowman-Birk protease inhibitor on survival of fibroblasts and cancer cells exposed to radiation and *cis*-platinum. Nutr. Cancer. 1996; 26:209–217. [PubMed: 8875558]

270. Gueven N, Dittmann K, Mayer C, Rodemann HP. Bowman-Birk protease inhibitor reduces the radiation-induced activation of the EGF receptor and induces tyrosine phosphatase activity. Intl. J. Radiat. Biol. 1998; 73:157–162. [PubMed: 9489562]

271. Gueven N, Dittmann K, Mayer C, Rodemann HP. The radioprotective potential of the Bowman-Birk protease inhibitor is independent of its secondary structure. Cancer Letters. 1998; 125:77–82. [PubMed: 9566699]

272. Kennedy AR, Ware JH, Guan J, Donahue JJ, Biaglow JE, Zhou Z, Stewart J, Vasquez M, Wan XS. Selenomethionine protects against adverse biological effects induced by space radiation. Free Rad. Biol. Med. 2004; 36:259–266. [PubMed: 14744637]

273. Wan XS, Ware JH, Zhou Z, Donahue JJ, Kennedy AR. Protection against radiation induced oxidative stress in cultured human epithelial cells by treatment with antioxidant agents. International Journal of Radiation Oncology, Biology, Physics. 2006; 64:1475–1481.

274. Guan J, Wan XS, Zhou Z, Ware JH, Donahue JJ, Biaglow JE, Kennedy AR. The effects of dietary supplement agents on space radiation induced oxidative stress in Sprague-Dawley rats. Radiation Research. 2004; 162:572–579. [PubMed: 15624312]

275. Guan J, Stewart J, Ware JH, Zhou Z, Donahue JJ, Kennedy AR. Effects of dietary supplements on the space radiation induced reduction in total antioxidant status in CBA mice. Radiation Research. 2006; 165:373–378. [PubMed: 16579649]

276. Kennedy AR, Guan J, Ware JH. Countermeasures against space radiation induced oxidative stress in mice. Radiat. Environ. Biophys. 2007; 46:161–165. [PubMed: 17265150]

277. Kennedy AR, Zhou Z, Donahue JJ, Ware JH. Protection against space radiation induced adverse biological effects by the Bowman-Birk inhibitor and antioxidants. Radiation Research. 2006; 166:327–332. [PubMed: 16881733]

278. Fahey RC. Protection of DNA by thiols. Pharmacology & Therapeutics. 1988; 39:101–108. [PubMed: 3059361]

279. Prise KM, Gillies NE, Whelan A, Newton GL, Fahey RC, Michael BD. Role of charge in the radioprotection of E. coli by thiols. International Journal of Radiation Biology. 1995; 67:393–401. [PubMed: 7738402]

280. Gencel O, Naziroglu M, Celik O, Yalman K, Bayram D. Selenium and vitamin E modulates radiation-induced liver toxicity in pregnant and nonpregnant rat: effects of colemanite and hematite shielding. Biological Trace Element Research. 2010; 135:253–263. [PubMed: 19763408]

281. Boldyrev AA. Protection of proteins from oxidative stress: a new illusion or a novel strategy? Annals of the New York Academy of Sciences. 2005; 1057:193–205. [PubMed: 16399895]

282. Ware JH, Zhou Z, Romero-Weaver AL, Wan XS, Newberne PM, Kennedy AR. Effects of selenomethionine in irradiated human thyroid epithelial cells and tumorigenicity studies. Nutrition & Cancer. 2011; 63:1114–1121. [PubMed: 21916697]

283. Mettler FA Jr, Voelz GL. Major radiation exposure--what to expect and how to respond. New England Journal of Medicine. 2002; 346:1554–1561. [PubMed: 12015396]

284. Koenig KL, Goans RE, Hatchett RJ, Mettler FA Jr, Schumacher TA, Noji EK, Jarrett DG. Medical treatment of radiological casualties: current concepts. Annals of Emergency Medicine. 2005; 45:643–652. [PubMed: 15940101]

285. Brown SL, Kolozsvary A, Liu J, Jenrow KA, Ryu S, Kim JH. Antioxidant diet supplementation starting 24 hours after exposure reduces radiation lethality. Radiation Research. 2010; 173:462–468. [PubMed: 20334518]

286. Matsumoto H, Hamada N, Takahashi A, Kobayashi Y, Ohnishi T. Vanguards of paradigm shift in radiation biology: radiation-induced adaptive and bystander responses. Journal of Radiation Research. 2007; 48:97–106. [PubMed: 17327685]

287. Matsumoto H, Tomita M, Otsuka K, Hatashita M, Hamada N. Nitric oxide is a key molecule serving as a bridge between radiation-induced bystander and adaptive responses. Current Molecular Pharmacology. 2011; 4:126–134. [PubMed: 21143183]

288. Durocher D, Jackson SP. DNA-PK, ATM and ATR as sensors of DNA damage: variations on a theme? Current Opinion Cell Biol. 2001; 13:225–231. [PubMed: 11248557]

289. Xu J, Xin S, Du W. Drosophila Chk2 is required for DNA damage-mediated cell cycle arrest and apoptosis. FEBS Letters. 2001; 508:394–398. [PubMed: 11728459]

290. Hirao A, Cheung A, Duncan G, Girard PM, Elia AJ, Wakeham A, Okada H, Sarkissian T, Wong JA, et al. Chk2 is a tumor suppressor that regulates apoptosis in both an ataxia telangiectasia mutated (ATM)-dependent and an ATM-independent manner. Mol. Cell. Biol. 2002; 22:6521–6532. [PubMed: 12192050]

291. Seo YR, Kelley MR, Smith ML. Selenomethionine regulation of p53 by a ref1-dependent redox mechanism. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:14548–14553. [PubMed: 12357032]

292. Rafferty TS, Beckett GJ, Walker C, Bisset YC, McKenzie RC. Selenium protects primary human keratinocytes from apoptosis induced by exposure to ultraviolet radiation. Clinical & Experimental Dermatology. 2003; 28:294–300. [PubMed: 12780718]

293. Husbeck B, Peehl DM, Knox SJ. Redox modulation of human prostate carcinoma cells by selenite increases radiation-induced cell killing. Free Radical Biology & Medicine. 2005; 38:50–57. [PubMed: 15589371]

294. Fischer JL, Lancia JK, Mathur A, Smith ML. Selenium protection from DNA damage involves a Ref1/p53/Brca1 protein complex. Anticancer Research. 2006; 26:899–904. [PubMed: 16619485]

295. Zhao R, Xiang N, Domann FE, Zhong W. Expression of p53 enhances selenite-induced superoxide production and apoptosis in human prostate cancer cells. Cancer Research. 2006; 66:2296–2304. [PubMed: 16489034]

296. Brash DE, Havre PA. New careers for antioxidants. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:13969–13971. [PubMed: 12391310]

297. Stewart J, Ware J, Fortina P, Breaux J, Gulati S, Kennedy A. L-selenomethionine modulates high LET radiation-induced alterations of gene expression in cultured human thyroid cells. Oncol. Rep. 2006; 16:569–574. [PubMed: 16865257]

298. Stewart J, Ko YH, Kennedy AR. Protective effects of L-selenomethionine on space radiation induced changes in gene expression. Radiation & Environmental Biophysics. 2007; 46:161–165. [PubMed: 17265150]

299. Sanzari JK, Nuth M, Kennedy AR. Induction of cytokine gene expression in human thyroid epithelial cells irradiated with HZE particles (iron ions). Radiation Research. 2009; 172:437–443. [PubMed: 19772464]

300. Neff RD, Cassen B. Relative radiation sensitivity of circulating small and large lymphocytes. Journal of Nuclear Medicine. 1968; 9:402–405. [PubMed: 5713816]

301. King G, Rabin B, Weatherspoon J. 5-HT3 receptor antagonists ameliorate emesis in the ferret evoked by neutron or proton radiation. Aviation, Space and Environmental Medicine. 1999; 70:485–492.

302. Levi M, Toh CH, Thachil J, Watson HG. Guidelines for the diagnosis and management of disseminated intravascular coagulation. British Committee for Standards in Haematology. British Journal of Haematology. 2009; 145:24–33. [PubMed: 19222477]

303. Bond VP, Robinson CV. A mortality determinant in nonuniform exposures of the mammal. Radiation Research - Supplement. 1967; 7:265–275. [PubMed: 6058663]

304. Morris MD, Jones TD. A comparison of dose-response models for death from hematological depression in different species. International Journal of Radiation Biology & Related Studies in Physics, Chemistry & Medicine. 1988; 53:439–456.

305. Hall, EJ.; Giaccia, AJ. Radiobiology for the Radiologist. Philadelphia: Lippincott, Williams & Wilkins; 2006.

306. Harding, RK. 5-HT3 receptor antagonists and radiation-induced emesis: preclinical data. In: Reynolds, DJM.; Andrews, PLR.; Davis, CJ., editors. Serotinin and the scientific basis of anti-emetic therapy. Oxford, London: Oxford Clinical Communications; 1995. p. 127-133.

307. Moroni M, Lombardini E, Salber R, Kazemzedeh M, Nagy V, Olsen C, Whitnall MH. Hematological changes as prognostic indicators of survival: similarities between Gottingen minipigs, humans, and other large animal models. PLoS ONE. 2011; 6:e25210. [PubMed: 21969873]

308. Moroni M, Coolbaugh TV, Lombardini E, Mitchell JM, Moccia KD, Shelton LJ, Nagy V, Whitnall MH. Hematopoietic radiation syndrome in the Gottingen minipig. Radiation Research. 2011; 176:89–101. [PubMed: 21520996]

309. Andersen AC. A substance observed within the vascular system of dogs receiving lethal exposures of whole-body x-irradiation. Radiation Research. 1957; 6:361–370. [PubMed: 13408478]

310. Winchell HS, Anderson AC, Pollycove M. Radiation-induced hemorrhagic diathesis in dogs unassociated with thrombocytopenia: association with an intravascular protein polysaccharide particle. Blood. 1964; 23:186–192. [PubMed: 14122243]

311. Liebow AA, Warren S, DeCoursey E. Pathology of atomic bomb casualties. American Journal of Pathology. 1949; 25:853–1027. [PubMed: 18147964]

312. Lushbaugh, CC. Advances in Radiation Biology. New York: Academic Press Inc.; 1969. Reflections on some recent progress in human radiobiology; p. 277-314.

313. Fujita S, Kato H, Schull WJ. The LD50 associated with exposure to the atomic bombing of Hiroshima and Nagasaki. Journal of Radiation Research. 1991; 32(Suppl):154–161. [PubMed: 1762100]

314. Reitan, JB.; Stavem, P.; Kett, K.; Hoel, PS. The ^{60}Co accident in Norway 1982: A Clinical Reappraisal. In: Ricks, RC.; Fry, SA., editors. The Medical Basis for Radiation Accident Preparedness. II Clinical Experience and Follow-up since 1979. New York: Elsevier Science Publishing Co.; 1990. p. 3-11.

315. Valverde, NJ.; Cordeiro, JM.; Oliveira, AR.; Brandao-Mello, CE. The acute radiation syndrome in the ^{137}Cs Brazilian Accident, 1987. In: Ricks, RC.; Fry, SA., editors. The Medical Basis for Radiation Accident Preparedness II. Clinical Experience and Follow-up since 1979. New York: Elsevier Science Publishing Co., Inc.; 1990. p. 89-107.

316. Rosenthal JJ, de Almeida CE, Mendonca AH. The radiological accident in Goiania: the initial remedial actions. Health Physics. 1991; 60:7–15. [PubMed: 1983986]

317. Lorenz E, Congdon CC. Radioactivity; biologic effects of ionizing radiations. Annual Review of Medicine. 1954; 5:323–338.

318. Miller CP, Hammond CW, Tompkins M. The role of infection in radiation injury. Journal of Laboratory & Clinical Medicine. 1951; 38:331–343. [PubMed: 14880746]

319. Eisele GR, West JL. Bacteriological evaluations of swine exposed to lethal levels of gamma radiation. Journal of Animal Science. 1973; 37:27–32. [PubMed: 4198388]

320. Eldred E, Trowbridge WV. Radiation sickness in the monkey. Radiology. 1954; 62:65–73. [PubMed: 13134493]

321. USAEC, U. S. Atomic Energy Commission. The Report of the Joint Commission for the Investigation of the Effects of the Atomic Bomb in Japan. Washington, D.C.: III. Department of Energy; 1951. Medical Effects of Atomic Bomb.

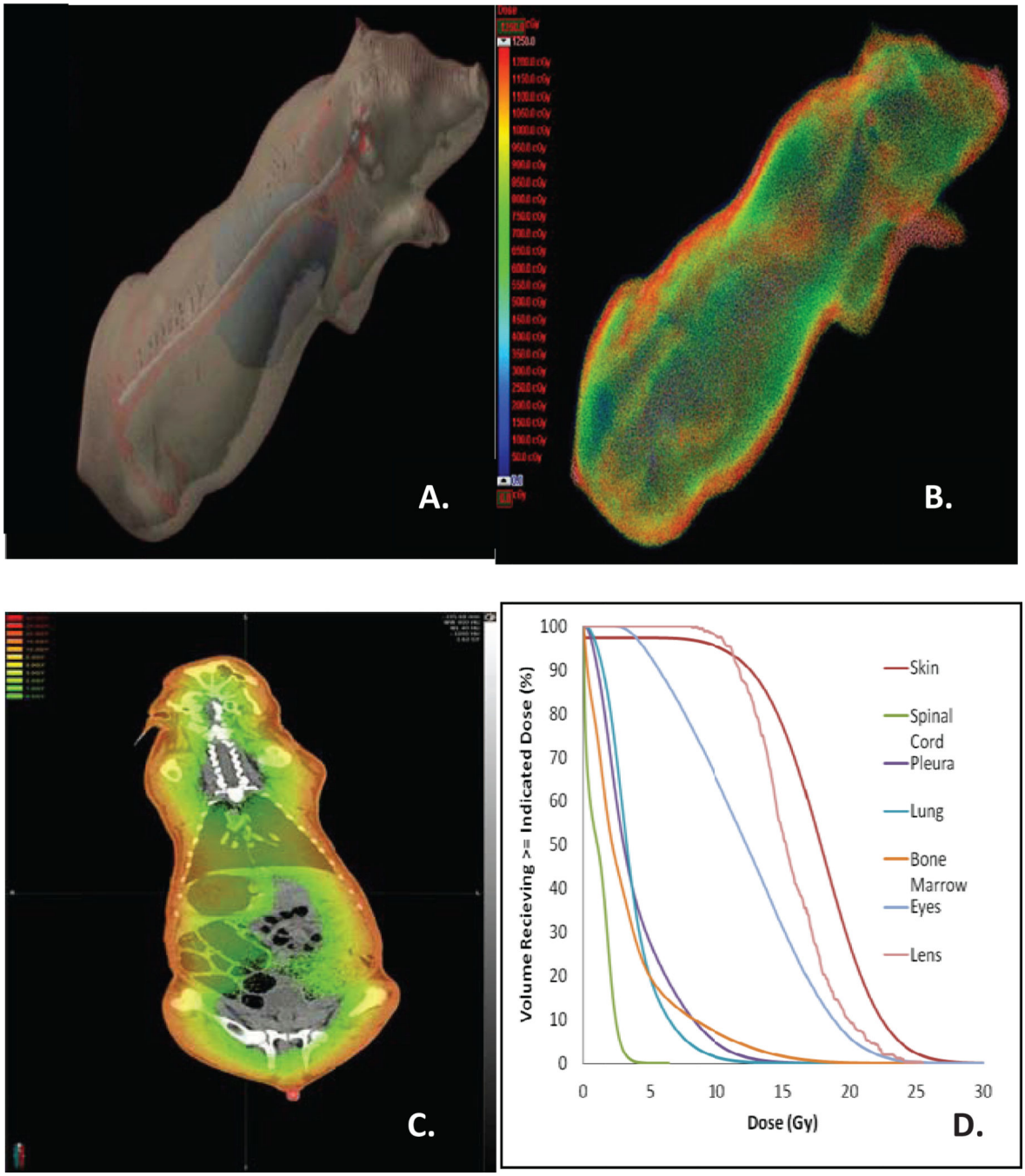

Figure 1.

Simulated dosimetry for Yucatan minipigs. Modern radiation oncology approaches, using CT based Monte Carlo dosimetry, have been incorporated into recent studies to accurately predict specific organ doses from SPE radiation exposure in animals. Pig data are shown in the figures. A: 3D reconstruction from a pig CT image with the skin rendered translucent to allow viewing of the internal organs (i.e., spinal cord [white], bone marrow [red], lung pleura [blue]). The organs were identified on separate CT axial cross sections generating a 3D organ volume from the combined axial contours; (This figure has been reproduced with

permission from Radiation Research (130)). B: Dose overlay on the 3D rendering of the whole body CT scan. (This figure has been reproduced with permission from Radiation Research (130)). C: Dose overlay on a pig CT image (coronal plane cross-section) for 6 + 12 MeV electron irradiation (often called a Heat map or a Dose-Color map) (Doses were simulated for 6 + 12 MeV electron irradiation of a pig using a Monte Carlo based simulation algorithm (Varian Medical Systems, Palo Alto, CA). D: Dose-volume histogram (DVH figure) illustrates organ radiation doses determined using the organ volumes and simulated dose distribution for the pigs receiving a skin dose of 20 Gy (from electrons). Note the high skin dose in the dose-volume histogram (DVH) figure and the fact that the lens and eye doses are also very high. (Images C and D – courtesy of Dr. Eric Diffenderfer)

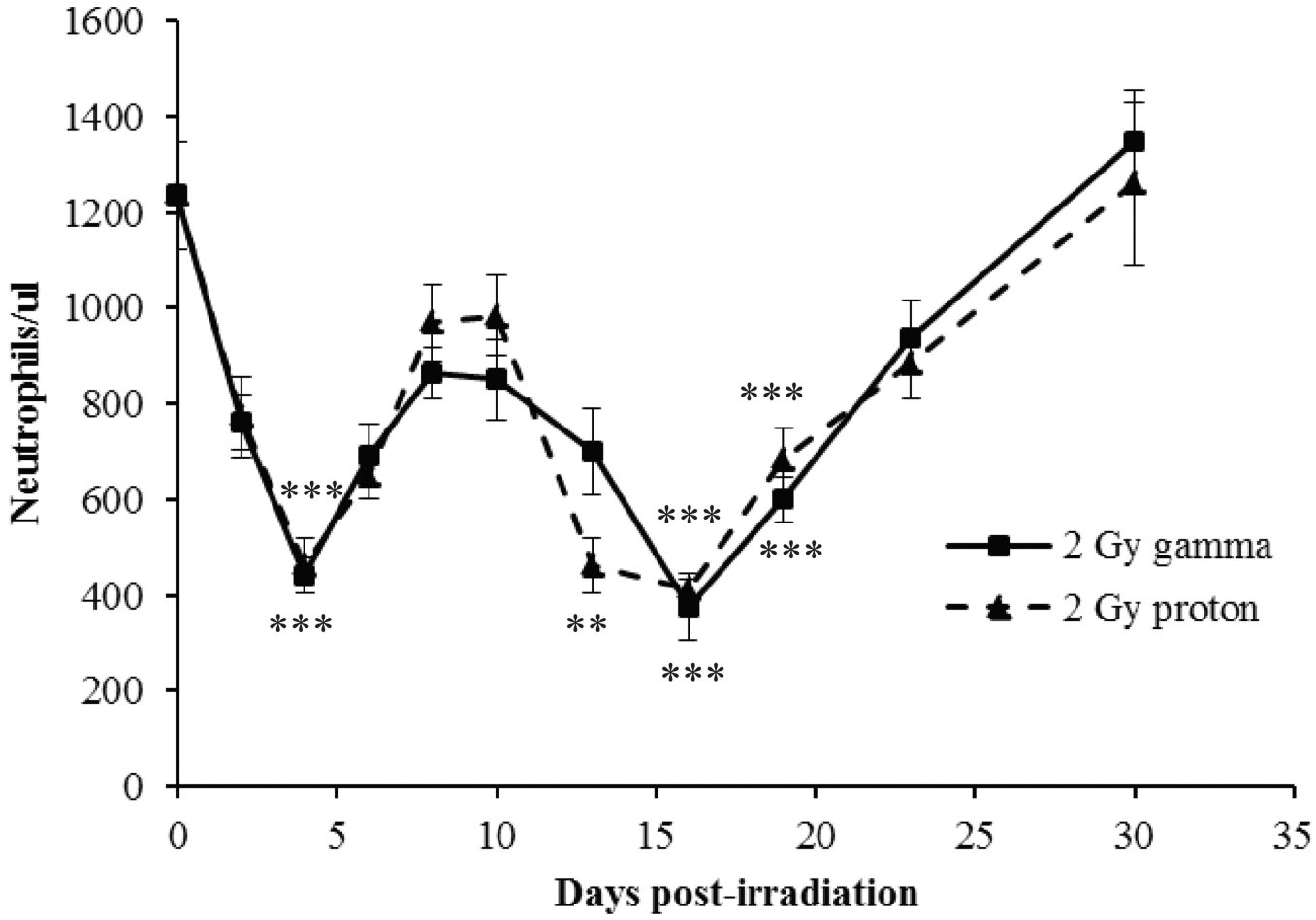

Figure 2.

Changes in neutrophil counts of mice exposed to a 2 Gy dose of SPE proton or γ-ray radiation. As shown in the figure, the effects of SPE like proton radiation on circulating neutrophil counts of mice are approximately the same as those of γ-ray radiation. In this figure, the absolute neutrophil counts are given at various times post-irradiation. In radiation therapy patients, when the white blood cell counts fall below the level of 500 cells/ microliter, it would trigger a medical response and the patients would be considered as candidates for countermeasures (e.g., Neulasta treatment). Thus, after irradiation of the mice with either SPE like proton or γ-ray radiation, the neutrophil counts fall to critically low values (<500 cells per microliter). (courtesy of Dr. Ana Romero-Weaver; data from Romero-Weaver et al. (145))

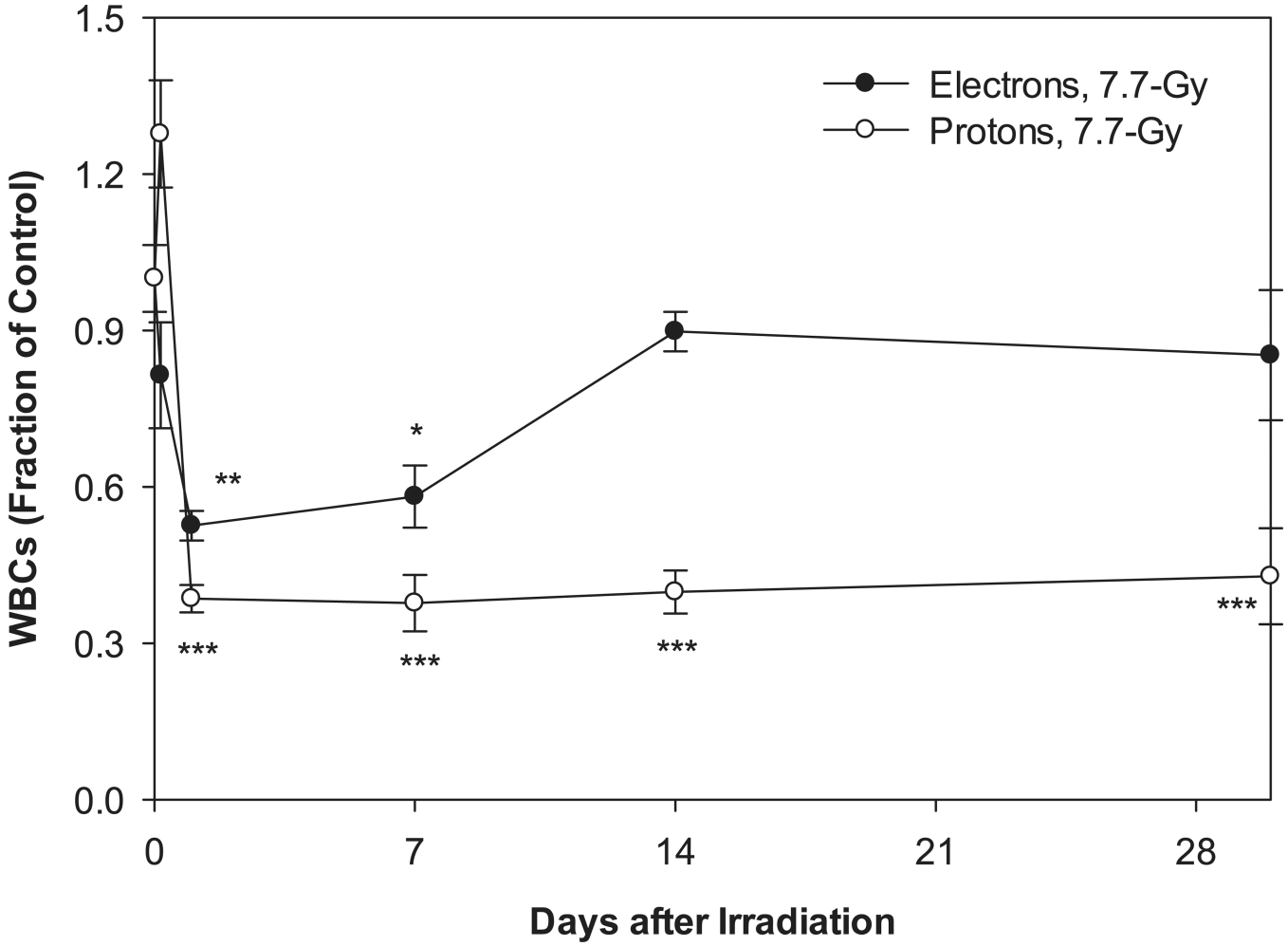

Figure 3.

Changes in WBC counts of pigs exposed to eSPE or pSPE radiation with a skin dose of 7.7 Gy. The pSPE dose involved several different energies of SPE radiation, as described previously (144), and the electron exposure involved two different energies of electrons (6 and 12 MeV electrons to result in a dose distribution like that expected for the SPE radiation exposure). The time point for these changes in the circulating white blood cell numbers was 30 days post-irradiation. It can be observed in the figure that pig white blood cells return to normal levels by 30 days post-exposure to electron radiation, but do not return to normal levels in proton irradiated pigs over the same time period. Significant difference as determined by one-way ANOVA followed by the Tukey Test is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

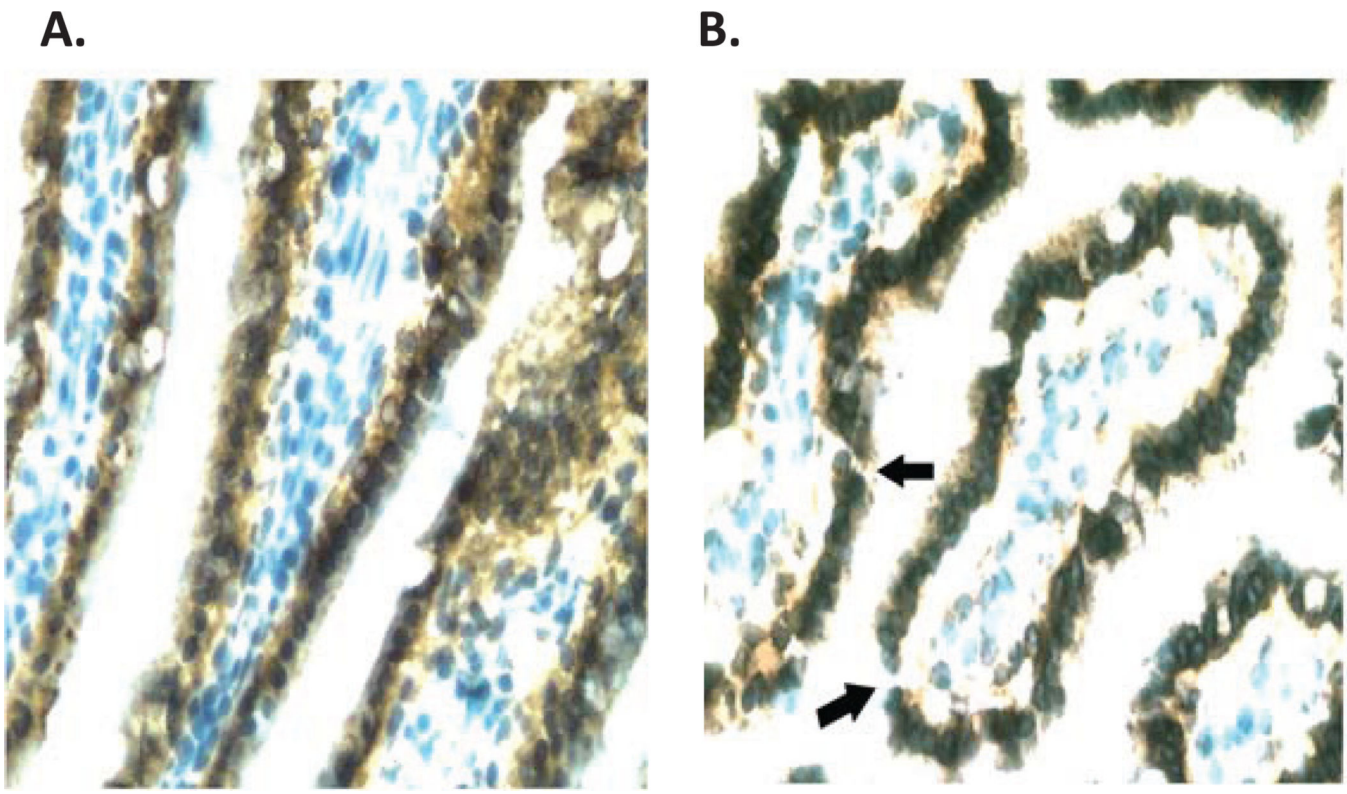

Figure 4.

A. Proton radiation induces breaks in the GI epithelial barrier. Terminal ileum obtained 2 days post-irradiation from a mouse irradiated with 2 Gy of protons at the low dose rate was stained for Claudin-3. Black arrows show regions of tight junction incongruity. Original magnification 400 ×. Reprinted with permission from Radiation Research (153).

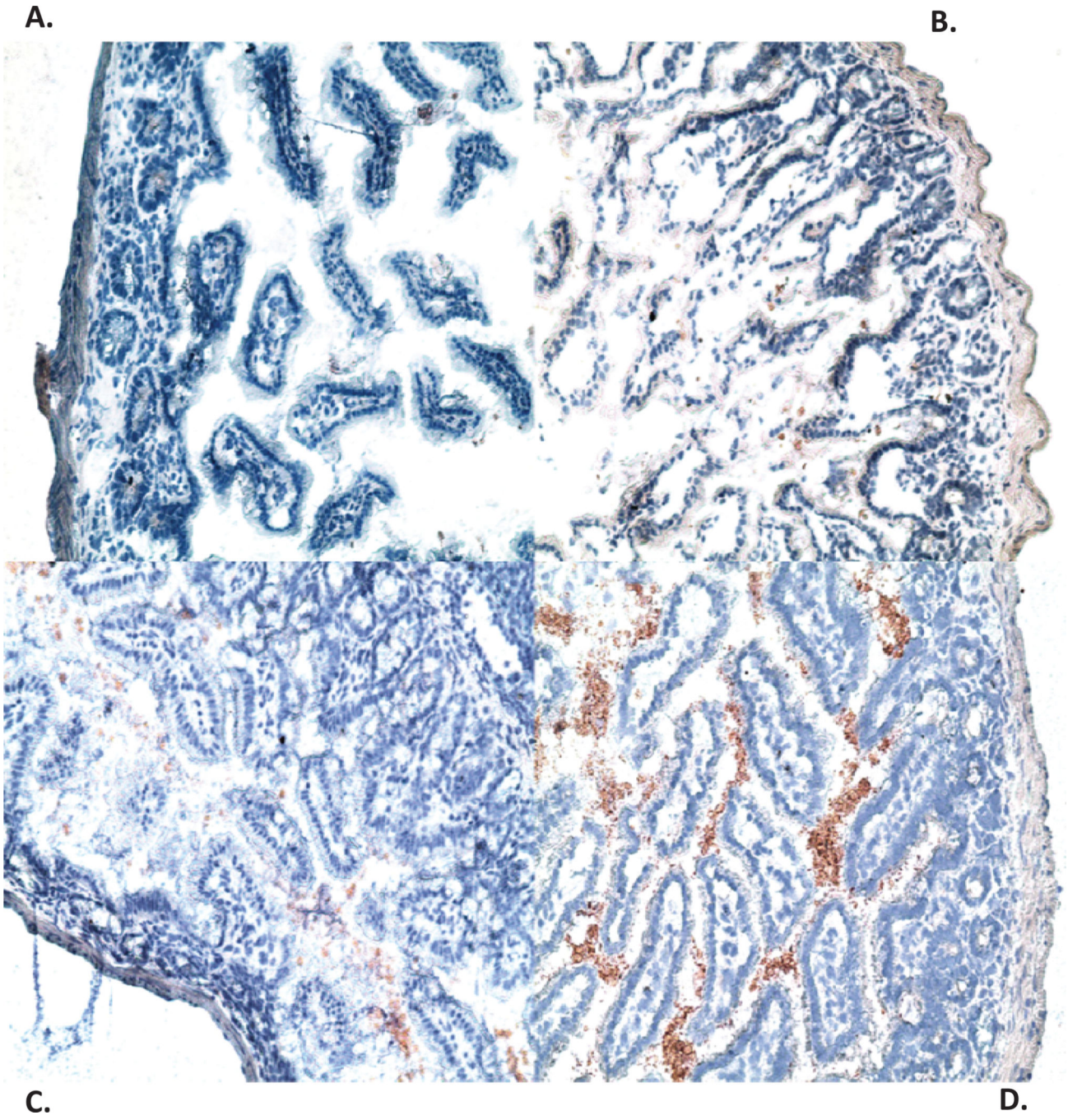

Figure 5.

SPE-like proton radiation and hindlimb suspension lead to the accumulation of LPS in subepithelial regions of the ileum. Terminal ileum obtained 4 days post irradiation and/or 6 days post hindlimb suspension or from control animals was stained for LPS using a mouse mAb specific for E.coli LPS. A: represents ileum from a control mouse, B: ileum for a mouse irradiated with 2 Gy of 70 MeV protons, C: ileum from a mouse subjected to hindlimb suspension, and D: ileum from a mouse subjected to HS and irradiated with 2 Gy of 70 MeV protons. It can be observed in the figure that the amount of LPS accumulated in

the subepithelial region of the ileum is considerably greater in the mouse exposed to both HS and SPE proton irradiation than in mice exposed to either HS or SPE proton radiation alone. Original magnifications – 200×.

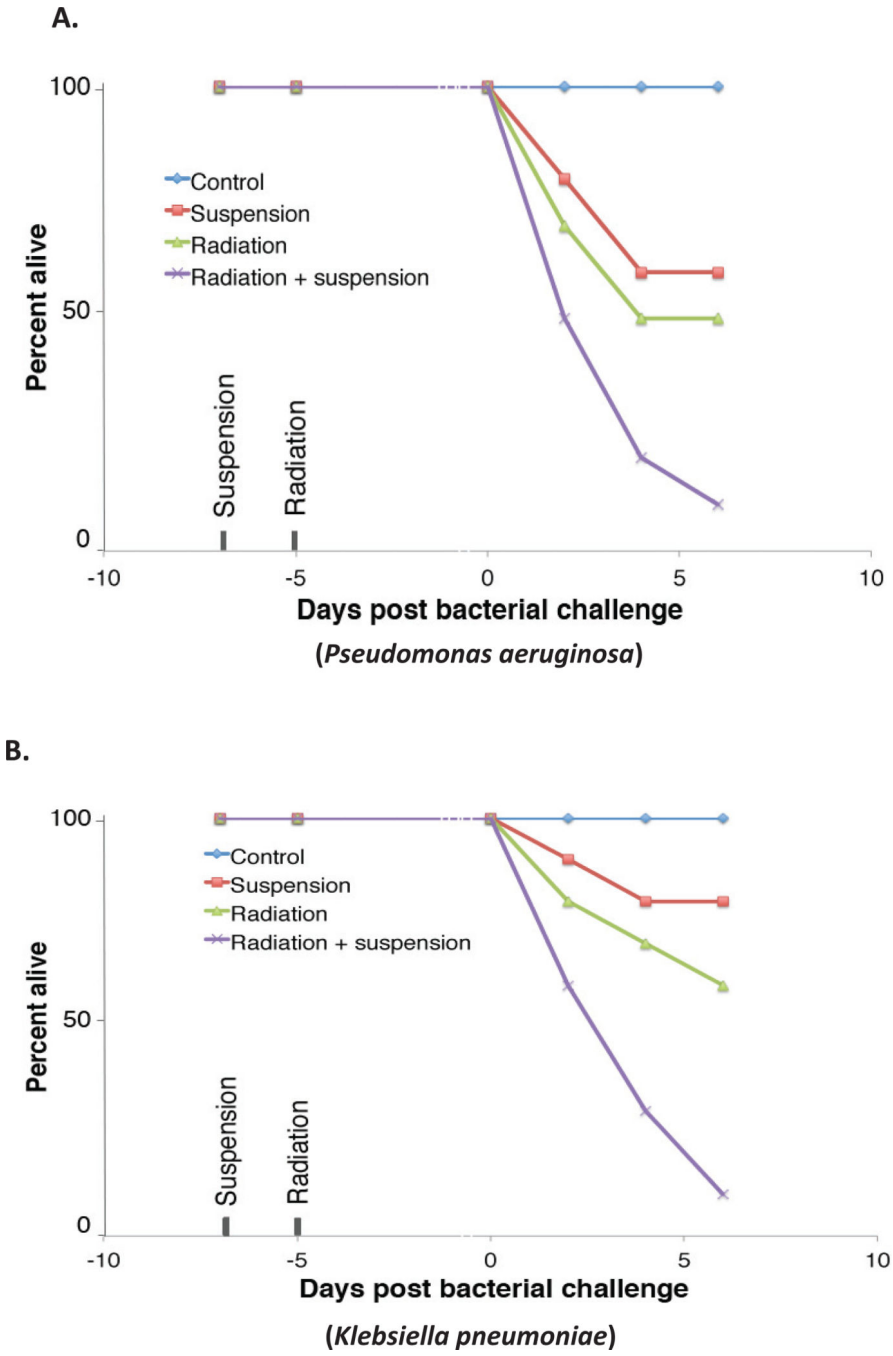

Figure 6.

Survival of mice challenged with bacteria after irradiation with or without hindlimb suspension. C3H/HeN mice were treated with HS, 2 Gy of SPE-like proton radiation, or both, and then the mice were challenged with a non-toxic dose of *Pseudomonas aeruginosa* (Panel A) or *Klebsiella pneumoniae* (Panel B) bacteria. There was a high level of mortality observed in all treatment groups except the control group; these mice were not exposed to HS or radiation, but were given the bacterial challenge. (The images were provided by Drew Weissman, M.D., Ph.D. [data from reference (151)].

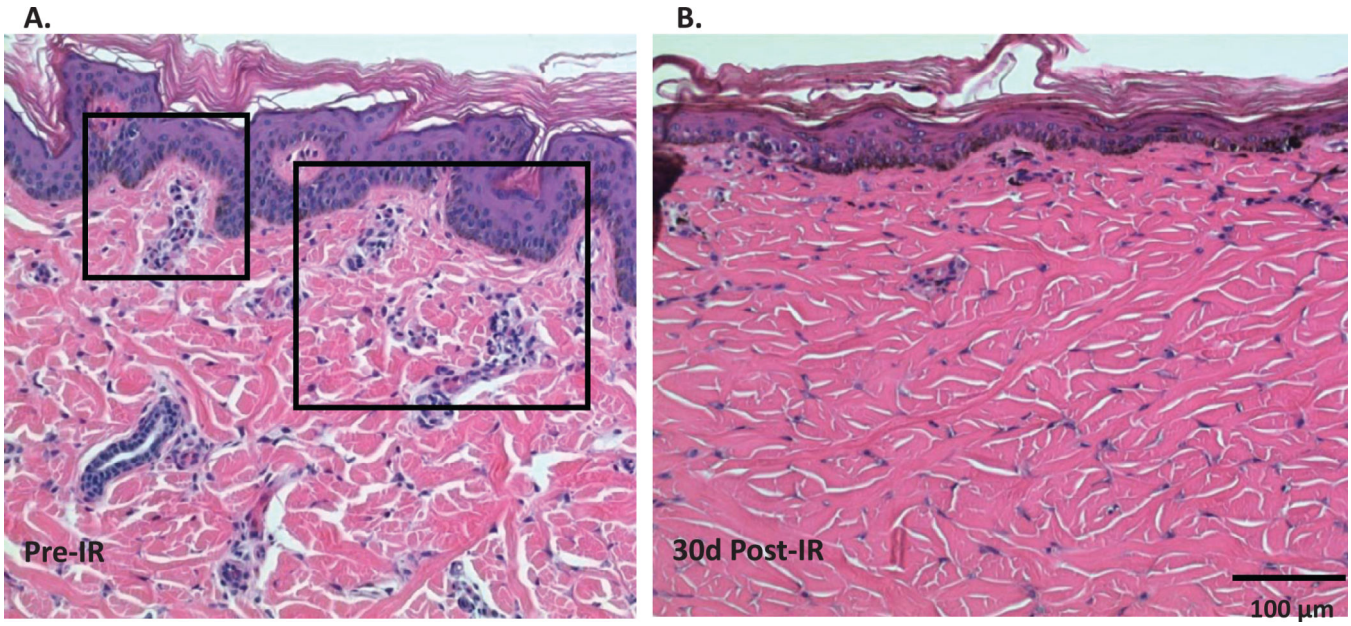

Figure 7.

Loss of blood vessels in the pig dermis after a high skin dose exposure to SPE-like radiation. Pig skin tissue samples were taken pre-irradiation (Panel A) and at 30 days after the pig was exposed to a 10 Gy skin dose of 6 + 12 MeV electron radiation (Panel B). For the skin tissue taken before irradiation, vascular beds are denoted by squares (Panel A). A consequence of exposure to the high skin dose from SPE radiation is that blood vessels disappear in the areas beneath the epidermis (Panel B), such that the blood flow to the cells in this area is reduced. Such areas of reduced blood supply beneath the epidermis occur in interventional radiology patients, and are known to be life-threatening (218, 219). Thus, the areas with reduced blood supply must be removed surgically.

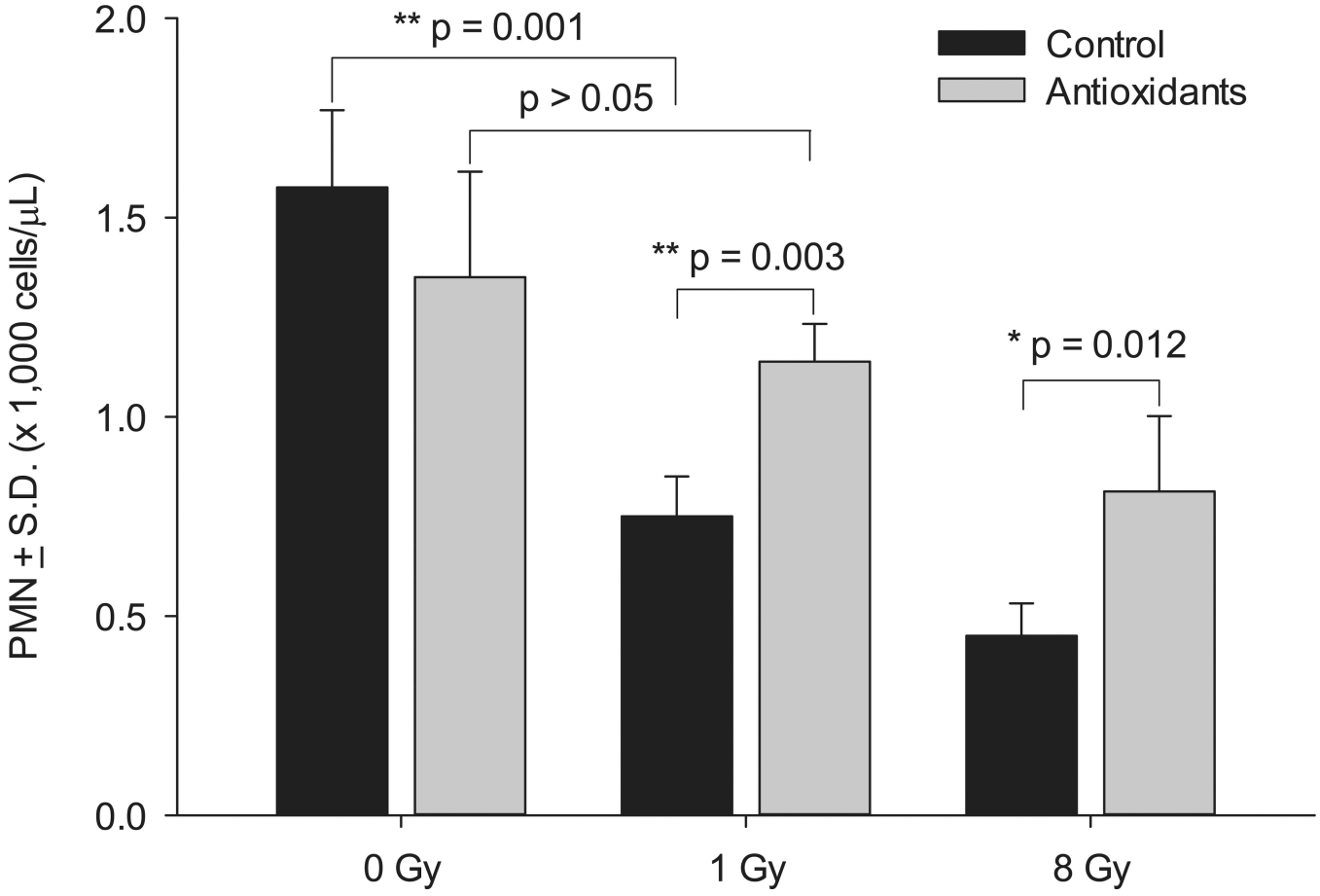

Figure 8.

Effects of radiation on polymorphonuclear/neutrophil counts in mice treated with antioxidants. In this experiment, animals were maintained on the control diet or antioxidant supplemented diet and irradiated with x-rays at doses of 1 or 8 Gy, which significantly decreased the numbers of circulating PMN cells (neutrophils) to a greater extent in mice maintained on the control diet than in mice maintained on the antioxidant supplemented diet. The magnitude of PMN loss in mice maintained on the antioxidant diets and exposed to 8 Gy of radiation was comparable to that observed in the mice maintained on the control diet and exposed to a 1 Gy dose of radiation. The decrease in the PMN count in mice maintained on the antioxidant supplemented diet and exposed to a 1 Gy dose of radiation was not significantly different from that in the control animals. These results indicate major beneficial effects of antioxidants on the survival of circulating PMNs/neutrophils following the radiation exposure. [These data are representative of those published previously (132)].