Radiation and Primary Response to Lipopolysaccharide: Bone Marrow-derived Cells and Susceptible Organs

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Abstract. Background: The major goal of this study was to determine whether radiation significantly alters bone marrow-derived cell distribution and mass of sensitive organs after challenge with lipopolysaccharide (LPS). Materials and Methods: C57BL/6 mice were exposed whole-body to 0 or 3 gray (Gy) Ï€-radiation (60Co) and injected intraperitoneally with 0.1 ml saline or 1 mg/kg LPS (E. coli serotype 0111:B4) 10 days later. Subsets from each group were euthanized at 60 min and 1, 7 and 14 days post-injection for analyses. Results: Body mass was low 1 day after LPS, especially in irradiated animals. LPS-induced splenomegaly and hepatomegaly were attenuated by radiation, whereas thymic atrophy was enhanced. However, radiation had no effect on LPS-induced changes in oxygen radical production by liver phagocytes. The numbers of all major leukocyte populations (lymphocytes, monocyte-macrophages, granulocytes) were altered by both radiation and LPS at virtually all time points of testing. In general, the LPS-induced changes in leukocytes were diminished by radiation. Significant radiation x LPS interactions were especially prominent at day 1 after LPS administration. In contrast, mice receiving both radiation and LPS had lower red blood cell (RBC) and platelet counts than those receiving either agent alone. Conclusion: The data show that radiation had a highly significant influence on LPS-induced changes in mass of several body organs, leukocytes, RBC, and platelets, and thus may increase severity of infection due to Gram-negative bacteria.

Radiation above normal background levels on Earth occurs at nuclear power plants, medical facilities, and during air and space travel. Although the radiation doses under these conditions are relatively low, much higher exposures can occur during long-term missions in space that include a solar particle event (SPE), accidents (e.g., Three Mile Island and Chernobyl), and nuclear terrorism (1-3). When given acutely, the lethal dose for 50% of humans at 60 days (LD50/60) after whole-body irradiation is between 3.25 and 4 gray (Gy) if there is little or no supportive care, and 6 to 7 Gy when antibiotics, blood transfusions and other support is provided (4). Leukopenia, anemia and thrombocytopenia are typical manifestations of acute radiation syndrome; infection and hemorrhaging are major causes of death. Furthermore, the increasing prevalence of multi-drug resistant bacteria in both hospital and community settings is likely to increase morbidity and mortality after a radiological terrorism event. A better understanding of radiation effects on bone marrow-derived cells (i.e. leukocytes, erythrocytes, and thrombocytes) is needed to help clarify their role in radiation-induced multi-organ failure and identify effective counter-measures to supplement current therapies (5).

The normal response to a bacterial infection involves both innate and adaptive immune mechanisms, e.g., phagocytosis by neutrophils and macrophages and cytokines produced by T helper (Th) and other cells. Phagocyte activity is more efficient if the bacteria are opsonized with antibodies secreted by B lymphocytes, certain acute-phase molecules produced by the liver (e.g., mannose-binding lectin) and breakdown components of complement. If the immune system is intact, clearance of most bacteria is accomplished within a few hours. On the other hand, a deficient, dysfunctional, or poorly-controlled response after radiation exposure can result in overwhelming bacterial infection, septic shock and death. During sepsis, death can occur due to multi-organ failure, tissue and/or organ hyperfusion, or hypotension (6). Despite appropriate antibiotic use and critical care therapy, very little improvement has been achieved in disease management; the mortality rate in intensive care units is within the 40% to 50% range (7).
Lipopolysaccharide (LPS), or endotoxin, is a surface component of all Gram-negative bacteria that induces a very rapid response, often within 60-90 min, against the invaders. LPS also accounts for many of the signs and symptoms associated with Gram-negative infections, including fever, leukopenia followed by leukocytosis, disseminated intravascular coagulation, and cachexia. The mechanism of LPS action is indirect, mediated primarily through cytokines such as tumor necrosis factor-α (TNF-α) secreted by activated macrophages in the spleen, liver, and other sites. *E. coli*, an extremely common inhabitant of the intestinal tract, is a leading cause of Gram-negative sepsis.

Our previous studies with the same animal model used here have demonstrated that various forms of radiation dramatically affect bone marrow-derived cell populations (8-13). This and the accompanying paper (14) are the first in a series of studies in which we evaluated immune and other responses to bacterial and viral components in whole-body irradiated mice. Our overall hypothesis was that radiation would significantly alter normal responsiveness to a potent immunogenic component of *E. coli*. We further proposed that radiation would cause anemia and thrombocytopenia, conditions that could increase complexity of patient management. In this report, the LPS serotype 0111:B4 was used to simulate *E. coli* infection. This serotype has been previously utilized as an inducer of sepsis in investigations of new treatment modalities (15). In addition, mutant forms of LPS 0111:B4 are currently in development as vaccines for both the prevention and treatment of sepsis (16). The 3 Gy dose of radiation we used throughout is within the range that could be experienced by survivors of significant radiation exposure.

Materials and Methods

**Animals.** Female C57BL/6 mice at 8–9 weeks of age (n=160; Charles River Breeding Laboratories, Wilmington, MA, USA) were acclimatized for 1 week and then assigned to the following groups: a) 0 Gy+Saline, b) 3 Gy+Saline, c) 0 Gy+LPS, and d) 3 Gy+LPS. Subsets from each group were euthanized in 100% CO2 in compliance with the most recent recommendations of the Veterinary Medical Panel on Euthanasia, at 60 min and on days 1, 7, and 14 after LPS injection (n=10 mice/group/time point). The irradiation, administration of LPS, and subsequent assessments were performed in two identical experiments and the results were pooled. This study was approved by the Institutional Animal Care and Use Committee.

**Radiation.** Whole-body irradiation was performed utilizing a retired therapy unit with a 60Co source (AECL Eldorado machine, Atomic Energy of Canada Ltd., Commercial Products Division, Ottawa, Canada) having 1.17 and 1.33 MeV energy and linear energy transfer (LET) of 0.267 keV/μm. Non-anesthetized animals were placed into aerated 3x3x6 cm polystyrene boxes; radiation was delivered to 50% of the mice in a single fraction of 3 Gy at a dose rate of ~0.8 Gy/min. Additional details of this protocol are available elsewhere (13, 17). All mice were observed daily for signs of ill health. The Institutional Animal Care and Use Committee (ACUC) approved this study.

**Immune challenge with LPS.** Ten days after irradiation, LPS from *E. coli* serotype 0111:B4 (Sigma Chemical Co., St. Louis, MO, USA) was diluted in phosphate buffered saline (PBS) and injected i.p. in a single dose of 1 mg/kg body mass and a volume of 0.1 ml/mouse. Control animals received vehicle without LPS. The LPS dose was within the range reported to induce significant immune modulation (18). Selection of the 10-day post-irradiation time point for immune challenge was based on data from our previous studies demonstrating that hematopoietic reconstitution was already well under way after a 3 Gy exposure (10).

**Body and normalized organ masses.** At the times of euthanasia, mice were weighed and the spleen, thymus, liver and lungs were excised and weighed. The organs were not flushed with saline to remove blood supply prior to weighing. The mass of each organ was normalized to body mass as follows: relative organ mass (ROM) = organ mass (mg)/body mass (g).

**Oxygen radical production by phagocytic cells in liver.** Oxygen radical production was quantified using portions of liver as previously described (19). This assay uses 2',7'-dichloro-dihydrofluorescein diacetate (DCFH-DA; Invitrogen, Corp., Carlsbad, CA, USA) as a probe and Zymosan A (Sigma) from yeast cell wall (*Saccharomyces cerevisiae*) as the triggering agent. Cells were plated into wells of 96-microwell plates at 2.5x10 6/well/0.1 ml and incubated for 1 h at 37°C in 5% CO2. DCFH-DA/Zymosan working solution (150 μM DCFH-DA in 20 mg Zymosan/ml Hanks Salt Solution) was added at 10 μl/well and the plates were reincubated for 1 h. Fluorescence intensity was measured with a fluorometer and expressed as relative fluorescence units (RFU).

**Hematological analysis.** Whole blood was collected in [K2]EDTA-containing syringes by cardiac puncture immediately after euthanasia. White blood cell (WBC), red blood cell (RBC), and platelet (PLT) counts, as well as the three-part differential (lymphocytes, monocyte-macrophages, and granulocytes) and eosinophil data were obtained with an automated analyzer (HESKA® Vet ABC-Diff Hematology Analyzer; Heska Corp., Fort Collins, CO, USA), as described elsewhere (12, 20). Additional important information provided by the analyzer included: hemoglobin concentration (HGB), hematocrit (HCT; percentage of whole blood composed of RBC), mean corpuscular volume (MCV; mean volume per RBC), mean corpuscular hemoglobin (MCH; mean weight of hemoglobin per RBC), mean corpuscular hemoglobin concentration (MCHC; mean concentration of hemoglobin per RBC), RBC distribution width (RDW), and mean platelet volume (MPV).

**WBC count and three-part differential in spleen.** Spleens were excised, dissociated into single-celled suspensions which were then filtered through 40 μm cell strainers (Becton Dickinson, Franklin Lakes, NJ, USA) to remove clumps and debris. The cells were washed once with medium, centrifuged and finally suspended in 2 ml of complete RPMI-1640 medium (Irvine Scientific, Santa Ana, CA, USA). The WBC and numbers of lymphocytes, monocyte-macrophages and...
granulocytes, as well as eosinophils, were obtained with the automated hematology analyzer as described above for blood.

Statistical analysis. Two-way analysis of variance (ANOVA) was performed using radiation and LPS as the independent variables.

Body mass. On day 1, radiation and LPS had main effects on body mass (p<0.005) and a radiation x LPS interaction was noted (p<0.001). The 0 Gy+LPS group had lower body mass compared to both irradiated groups (p<0.05) and the 3 Gy+LPS group had lower mass compared to all other groups (p<0.001). The means (g) on day 1 were: 20.7±0.3 (0 Gy+Saline), 21.0±0.2 (3 Gy+Saline), 19.8±0.2 (0 Gy+LPS), and 17.5±0.4 (3 Gy+LPS). Body masses were similar among groups at the other time points.

Relative organ mass. Figure 1 presents the spleen, thymus, liver, and lung masses when normalized to body mass. LPS increased spleen mass, with main effects seen at days 1, 7, and 14, whereas radiation ameliorated this response; a radiation x LPS interaction occurred on day 1. Thymus mass was reduced by radiation; a radiation x LPS interaction occurred at 60 min and a main radiation effect was seen on day 1. LPS had main effects on liver mass at days 1, 7, and 14, whereas radiation effects were seen only on day 14, at which time a radiation x LPS interaction occurred. Post hoc analysis showed that liver mass was enhanced by LPS at day 1 in 0 Gy mice and at day 14 in 3 Gy mice.

Oxygen radical production. As shown in Figure 2, LPS had no effect on oxygen radical production by phagocytic cells in the
Figure 3. WBC and major leukocyte populations in blood. Means±SEM were obtained using an automated hematology analyzer. WBC: p<0.005 for main effect of radiation at all time points; p<0.05 for effect of LPS at 60 min and 14 days. Lymphocytes: p<0.001 for effect of radiation at all time points; p<0.001 for effect of LPS at 1 day; p<0.001 for radiation x LPS interaction at 1 day. Monocytes: p<0.005 for effect of radiation at all time points; p<0.05 for effect of LPS at all time points; p<0.05 for radiation x LPS interaction at 1 day. Granulocytes: p<0.001 for effect of radiation at all time points; p<0.05 for effect of LPS at all time points; p<0.005 for radiation x LPS interaction at 1 day. a: p<0.001 vs. all other groups; b: p<0.001 vs. 0 Gy+LPS and 0 Gy+Saline; c: p<0.005 vs. 0 Gy+LPS and 0 Gy+Saline; d: p<0.05 vs. 0 Gy+LPS; e: p<0.05 vs. 0 Gy+LPS and 0 Gy+Saline; f: p<0.05 vs. 0 Gy+LPS and 0 Gy+Saline; g: p<0.05 vs. 0 Gy+Saline; h: p<0.05 vs. 0 Gy+Saline and 0 Gy+LPS; i: p<0.05 vs. 0 Gy+Saline and 0 Gy+LPS; j: p<0.005 vs. 0 Gy+Saline; k: p<0.005 vs. 3 Gy+LPS.

Figure 4. WBC and major leukocyte populations in spleen. Means±SEM were obtained using an automated hematology analyzer. WBC: p<0.01 for main effect of radiation at 60 min and 1 day; p<0.05 for effect of LPS at 1 day; p<0.01 for radiation x LPS interaction at 1 day. Lymphocytes: p<0.05 for effect of radiation at 1, 7, and 14 days; p<0.05 for radiation x LPS interaction at 1 day. Monocytes/macrophages: p<0.05 for effect of radiation at 60 min and 1 day; p<0.05 for effect of LPS at 7 days; p<0.005 for radiation x LPS interaction at 1 day. Granulocytes: p<0.05 for effect of radiation at 1 day; p<0.05 for effect of LPS at 7 days; p<0.005 for radiation x LPS interaction at 1 day. a: p<0.01 vs. 0 Gy+LPS and 0 Gy+Saline; b: p<0.05 vs. 3 Gy+LPS and 3 Gy+Saline; c: p<0.001 vs. 0 Gy+LPS and 0 Gy+Saline; d: p<0.05 vs. 3 Gy+LPS and 3 Gy+Saline; e: p<0.005 vs. 0 Gy+LPS and 0 Gy+Saline; f: p<0.05 vs. 0 Gy+LPS and 0 Gy+Saline; g: p<0.05 vs. 0 Gy+Saline and 3 Gy+LPS; h: p<0.05 vs. 0 Gy+Saline; i: p<0.05 vs. 0 Gy+Saline and 0 Gy+LPS; j: p<0.005 vs. 0 Gy+Saline; k: p<0.005 vs. 3 Gy+LPS; l: p<0.05 vs. 0 Gy+Saline.
liver at either 60 min or day 14. However, on day 1, there was a main effect of LPS and a radiation x LPS interaction. In general, radiation and LPS tended to decrease oxygen radical production, but statistical significance was obtained only for 0 Gy+LPS compared with 0 Gy+Saline.

Leukocyte populations in blood. Figure 3 presents the WBC, lymphocyte, monocyte and granulocyte counts in blood circulation. LPS had a main effect on the WBC at 60 min and 14 days; counts were decreased at 60 min compared to 0 Gy+Saline, but recovered thereafter. Radiation had main effects at all time points, resulting in relatively low WBC numbers. Lymphocytes were affected by LPS on day 1, whereas radiation had main effects at all time points. On day 1, lymphocytes were depleted in the 0 Gy+LPS group, but were low at all times of testing in both irradiated groups. LPS injection resulted in low monocyte counts at the two early times, a condition that was exacerbated when combined with radiation. However, on days 7 and 14, monocytes were lowest in the 0 Gy+Saline group. LPS reduced granulocytes at 60 min, but by day 1, the 0 Gy+LPS group had a higher count compared to all other groups. A radiation-induced decrease in granulocytes was seen only on day 14 in the 3 Gy+LPS group when compared to 0 Gy+Saline. There were Radiation x LPS interactions for all three major leukocyte types on day 1. In general, this was due to radiation-induced cell depletion, resulting in an attenuated response to LPS. Within the granulocyte population, there was a dramatic increase in eosinophils on day 1 after LPS, whereas radiation ameliorated this response, resulting in a Radiation x LPS interaction at 60 min and day 1 (Figure 3).

Leukocyte populations in spleen. Figure 4 shows the WBC, lymphocyte, monocyte-macrophage and granulocyte counts in the spleen. LPS had main effects on the WBC at 14 days and radiation effects occurred at 60 min and days 1 and 7; the 0 Gy+LPS group had the highest counts and differed from one or both irradiated groups on days 1, 7, and 14. Irradiated mice generally had the lowest WBC counts; significantly low numbers were noted for both 3 Gy groups at 60 min and the 3 Gy+LPS group at 1 day, at which time a radiation x LPS interaction was noted.

Radiation had main effects on lymphocyte counts at days 1, 7, and 14 due to cell depletion and a radiation x LPS interaction was observed on day 1. Monocyte-macrophages were increased in response to LPS; the 0 Gy+LPS group had high numbers on days 1 and 7. Radiation had main effects on monocyte-macrophages at 60 min and day 1. The 3 Gy+Saline group had lower numbers at day 1 compared to both non-irradiated groups and a radiation x LPS interaction was noted. There were main effects of LPS on splenic granulocytes at 7 and 14 days; subsequent analysis indicated that the 0 Gy+LPS group had increased counts on days 1 and 7. Radiation had a main effect on granulocytes at 1 day, with the 3 Gy+LPS group having the lowest counts, resulting in a radiation x LPS interaction at this time point. Within the granulocyte population, radiation caused an increase in eosinophils at 60 min regardless of LPS (Figure 5). High numbers were noted at days 1 and 7 for the 0 Gy+LPS group and at day 14 for the 3 Gy+LPS group when compared to one or more of the other groups. There was a radiation x LPS interaction on day 1.

Erythrocyte characteristics. RBC counts, HGB, and HCT are shown in Figure 6. Radiation resulted in significantly low RBC at 60 min and day 1 in both irradiated groups. On day 14, RBC were low only in the 3 Gy+LPS group. Similar patterns were observed for HGB and HCT. There were radiation x LPS interactions for all three parameters at 60 min due to an LPS-induced enhancement in the irradiated animals. Values obtained for additional erythrocyte characteristics are presented in Table I. Main effects of LPS were as follows: MCV at 1 and 14 days (decreased followed by increased values); MCHC at 1 day (increased values);
and RDW at 1, 7, and 14 days (decreased values followed by increases at the latter two time points). Radiation also had main effects: MCV at 7 and 14 days (increased values); MCH at 60 min and 1 and 7 days (increased values); and RDW at all time points (increased values). A radiation x LPS interaction was noted for MCH at 60 min.

Platelet characteristics. Both LPS and radiation had highly significant main effects on PLT counts at all time points (Figure 7). The LPS-induced decreases at 60 min and 1 day were followed by increases at 7 and 14 days in non-irradiated mice. Radiation reduced the PLT regardless of LPS at the three early time points. By day 14, PLT in the 3 Gy + LPS group had returned back to normal, whereas the 0 Gy + LPS mice had a higher count than all other groups. Radiation x LPS interactions occurred in PLT at all time points except day 14. LPS had a main effect on platelet volume (MPV) at 1 day and radiation had effects at all time points (Figure 7). Radiation x LPS interactions occurred on days 1 and 7. The pattern for MPV was generally opposite of that found for PLT counts, i.e. the lower the counts, the higher the volume.

Discussion

The data show a significant, although transient, LPS-induced loss in body mass that was especially striking in irradiated mice. LPS is a potent activator of macrophages that secrete TNF-α, a lipolytic cytokine that causes cachexia and interferes with production of adipose tissue (21). TNF-α expression is also induced by ionizing radiation (22). Hence,
the early and dramatic weight loss in irradiated animals receiving LPS may be related to enhanced TNF-α production by macrophages responding to radiation-induced tissue damage, as well as by radiation-induced activation of the TNF-α gene. By day 7, body mass was fully recovered and there were no deaths or other overt signs of toxicity due to either LPS or radiation exposure at any time.

The high radiosensitivity of lymphoid organs is manifested as atrophy during the first few days after exposure to doses such as 3 Gy (13, 17, 20, 23). Our data show that full recovery in spleen and thymus mass had already taken place at the time of LPS injection (i.e., 10 days post-irradiation). Lack of spleen enlargement in irradiated mice suggests a suboptimal response to LPS. Since the spleen is the major filter organ for antigens circulating in the blood (including LPS), compromised responsiveness could lead to slow resolution of infection with Gram-negative bacteria and possibly also increase risk for septic shock. In contrast to the spleen, LPS alone had no effect on thymus mass. However, when LPS was introduced into irradiated mice, there was transient thymic atrophy, a condition that occurs often during septic shock. The results indicate that radiation exacerbated LPS effects on thymus mass, possibly by induction of cytokines (e.g., TNF-α), corticosteroids, and leukemia inhibitory factor, all of which facilitate lymphocyte apoptosis (18, 24, 25).

During septic shock, multi-organ failure frequently involves the liver and lungs. Our data show no significant changes in lung mass. In contrast, LPS administration resulted in hepatomegaly regardless of radiation exposure, although significance was not obtained at all time points. The liver detoxifies many potentially harmful substances, including LPS, and removes waste products from the blood. Liver enlargement during sepsis is related to hepatocyte apoptosis and leukocyte infiltration (6, 26). These processes involve LPS-induced up-regulation of interactions among Kupffer cells, hepatocytes, and endothelial sinusoidal cells (6, 27). Our data show that generation of unstable oxygen radicals was decreased in livers from non-irradiated mice 1 day after LPS injection, but only when compared to the non-irradiated, saline-injected control group. These results suggest that a 3 Gy dose may have little or no effect on phagocyte response to LPS.

Radiation and LPS induced numerous changes in distribution of all major leukocyte populations in both the blood and spleen. In many cases, the effect of LPS was diminished in irradiated animals. The data also show that radiation-induced depletion of lymphocytes in the blood was still evident at 24 days post-exposure (i.e., day 14 after LPS injection). Although lymphocytes are not considered to be a primary defense against bacteria, B-cells produce opsinizing antibodies against bacterial antigens and T helper cells (i.e., Th1 subset) secrete interferon-γ (IFN-γ), a potent activator of macrophages. The low lymphocyte counts and attenuated response to LPS in irradiated animals suggest that severity of bacterial infections may be increased. Lymphocyte-mediated protection against other infectious agents such as viruses may also be suboptimal. The data in the accompanying report show a number of significant changes in the distribution and function of specific lymphocyte populations (14).

The lowest numbers of monocytes and macrophages were generally found in irradiated mice, especially in the spleen 1 day after LPS injection. Cells of this lineage express CD14, the receptor for LPS, and are its primary targets. Binding of LPS to CD14 signals production of TNF-α and other pro-inflammatory cytokines. The phagocytic ability of the cells is also increased, resulting in more rapid removal of particulate materials, thereby helping to resolve inflammation and maintain homeostasis. In addition, macrophages that have ingested apoptotic cells secrete a leukocyte protease inhibitor that down-regulates inflammation (28). Our data show that radiation may attenuate these normal responses due to reduced numbers of monocyte-macrophages.
Neutrophils, the numerically dominant granulocytes in the blood, are a front-line defense against bacteria; they rapidly migrate to the site of invasion, ingest, and destroy the microbes via degradative enzymes and oxygen radicals. LPS administration resulted in an initial reduction in granulocytes, with subsequent granulocytosis and an eventual return to a normal level. This sequence of events is likely due to rapid cell migration into tissues and organs in response to chemotactic signals, followed by a transient over-compensatory surge from the bone marrow into the blood. However, since irradiated mice receiving LPS did not exhibit this pattern, the data suggest that the number and/or the chemotactic response of granulocytes stored within the bone marrow was compromised.

Eosinophils (like neutrophils and monocyte-macrophages) express the CD14 receptor for LPS, migrate into organs in response to chemotactic signals and are rapidly replenished by an influx from the bone marrow (29). The data show that the LPS-induced eosinophilia was attenuated in irradiated animals and that radiation alone resulted in a high number of eosinophils in the spleen during the two early time points. High eosinophil counts during asthmatic and allergic inflammation have long been recognized. More recently, it has been demonstrated that eosinophils can store and secrete cytokines (e.g., IL-4, IL-13, TGF-β) and chemokines (e.g., RANTES), and are even capable of presenting antigen to lymphocytes (30). The health implications of the radiation/LPS-induced perturbations in eosinophils remain to be determined.

Anemia is often seen in astronauts (31) and patients with acute radiation syndrome (4). Our data show that RBC, as well as HGB and HCT, were dramatically reduced by LPS and that radiation exacerbated the effect. Radiation causes RBC shrinkage, lipid oxidation, and changes in transmembrane lipids, all of which indicate apoptosis (32). Furthermore, exposure to even low doses, such as 0.25 Gy to 1 Gy, results in transformation of RBC into echinocytes (33) that are susceptible to destruction and sequestration in small sinuses, thus resulting in low circulating numbers. Although hemoconcentration might be expected due to LPS-induced capillary leak syndrome, this was not observed under the present conditions. The findings indicate that the severity of anemia may be increased, at least for a period of time, in infected individuals who have also been exposed to radiation.

Radiation also amplified the decrease in platelets in mice given LPS. Presence of LPS results in a rapid accumulation of platelets in a variety of organs (i.e., lungs, liver, kidneys, spleen), resulting in low numbers in blood circulation (34). LPS-induced secretion of TNF-α also contributes to thrombocytopenia by directly triggering apoptosis and causing release of platelet agonists (35). In addition, LPS can cause disseminated intravascular coagulation, characterized by widespread thrombosis that can block capillaries, leading to hemorrhaging, shock and death. Mechanisms by which radiation causes thrombocytopenia include activation of TNF-α expression (22) and platelet adherence to damaged vasculature within kidneys and other organs (36). Since platelets are involved in tissue repair and wounds heal more slowly after irradiation (37), the incidence of LPS-associated hemorrhagic complications may be substantially increased in infected persons.

Conclusion

Collectively, the presented data show that whole-body exposure to a 3 Gy dose of radiation can alter leukocyte, erythrocyte and platelet parameters, as well as mass of organs that are critical in mediating resolution of Gram-negative bacterial infections. These are important findings especially when considered in the context of long-term space missions, during which astronauts have limited access to medical care, and terrorist acts that may combine release of radioactive materials with dispersal of infectious microorganisms.

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