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Whole-body Irradiation and Long-term Modification of Bone Marrow-derived Cell Populations by Low- and High-LET Radiation

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Abstract. *Background: The major aim of this study was to quantify long-term changes in bone marrow-derived cell populations after exposure to radiations of differing quality. Materials and Methods: Mice were whole-body irradiated to 2 Gy gamma, proton, carbon or iron radiation, and euthanized ~110 days later for immunocyte phenotyping. Results: Splenic lymphocytes and mono/macrophages increased after γ-rays when compared to 0 Gy and one or more of the other groups. There were high T cells (carbon vs. 0 Gy), high B cells (γ-rays vs. 0 Gy), and low natural killer (NK) cells (proton and carbon vs. 0 Gy). All radiations, except γ-rays, increased CD62L$^+$ memory T cell counts, whereas CD62L$^+$ B cells increased only after γ-rays. Conclusion: There were significant aberrations in many immune parameters nearly 4 months after exposure to various forms of radiation. This suggests radiation exposure can have long-term health consequences.*

There has been a steady escalation in the presence of humans in space over the past several decades and despite the tragic Columbia accident in 2003 (1), the exploration of space will inevitably continue. Current models of human space exploration include building outposts on the moon, paving the way for missions to Mars and beyond (2). With this increase in spaceflight activity, there will inevitably be an increase in exposure to radiation. However, current projections of health risks for exploration missions are highly uncertain, due, in part, to limited data on late effects of heavy particle radiation.

Two major sources of space radiation relevant to missions beyond Earth's orbit are: solar particle events (SPE) generated by our sun, and galactic cosmic rays (GCR) emanating from sources outside our solar system. Hypothetical models of a mission to Mars have suggested that astronauts may receive maximum cumulative doses ranging from 1 to 3 gray (Gy), depending upon shielding conditions (3-6). GCR and quiescent solar activity are estimated to contribute up to 1 sievert (Sv) dose equivalent to the total cumulative dose (4, 7). This low-dose/low-dose-rate exposure is fairly constant and predictable. In contrast, SPE are currently unpredictable and relatively acute, occurring over a matter of days. Worst-case models, based on the maximum fluence and energy spectra of three large events observed in 1956, 1972 and 1989, suggest that SPE will account for most of the estimated exposure dose (up to 2 Sv) (8). Recent analyses of ice core samples have indicated that the Carrington flare of 1859 was among the largest SPE in the past 500 years (9). Although the analysis was based primarily on estimates of the proton fluence for energies >30 MeV, the investigators concluded that the estimated, relatively acute doses would have been life threatening to astronauts, unless substantial shielding is provided (9). Additionally, a series of SPE reached Earth in fairly rapid succession in the fall of 2003, including one of the largest recorded events to date in terms of energy released, fluence and dose (10, 11). These new data will likely result in changes in future models, but it is unlikely that the expected dose due to SPE will fall below current estimates.

According to the National Aeronautics and Space Administration's (NASA) Strategic Program Plan for Space Radiation Health Research, ~90% of the dose equivalent in SPE is due to protons. The rest of the dose environment consists of other radiation species, including high Z and energy (HZE, *i.e.,* >100 MeV/nucleon) particles, such as iron, oxygen, carbon and silicon (12). These HZE particles are densely ionizing, deposit more than 50% of their energy along their linear tracks, have significantly greater ionizing power, and possess greater potential for causing damage (13-15). Even

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though only ~2% of the fluence is HZE particles, they are predicted to account for much of the biological consequences (4, 16-18), due to their high linear energy transfer (LET) and the characteristics of their track structure. Unfortunately, adequate shielding against these highly energetic and penetrating particles is currently impractical (19).

The hematopoietic system is exquisitely sensitive to radiation, and significant bone marrow depletion can occur after exposure to whole-body doses less than 1 Gy. Deaths may occur in some individuals after a single high-dose rate irradiation to doses as low as 1.2 Gy (20). Leukopenia, anemia and thrombocytopenia are typical acute clinical manifestations. Leukocytes, critical for an effective defense against microbial agents, are generally radiosensitive, making infection an important cause of death post-exposure. Leukocytes are also important in destruction of neoplastic cells, as evidenced by numerous attempts to up-regulate anti-tumor immunity (21).

There are relatively few reports describing the impact of charged particle exposure on the intact mammalian immune system. In previous *in vivo* studies, the relative susceptibility among specific lymphocyte phenotypes to acute ^{56}Fe whole-body irradiation was found to be similar to that of low-LET radiation on day 4 post-exposure, *i.e.*, B>T>natural killer (NK) and T cytotoxic (Tc) >T helper (Th) cells; dose-dependent increases were noted in the blood lymphocyte and B cell counts and the Th:Tc ratio in the spleen on day 113 (22, 23). There is, indeed, abundant evidence that radiation quality can make a big difference in the degree and type of cell damage, although most of these studies have been performed *in vitro* (13, 14, 19, 24-26).

In this study, the characteristics of specific leukocyte populations in mice nearly 4 months after whole-body exposure to ^{60}Co γ-rays, protons, carbon and iron ions are reported for the first time. Spleen, thymus, liver and lung masses in relation to body mass were also quantified to determine whether exposure to radiations encountered in space induces chronic effects in these highly radiosensitive organs.

Materials and Methods

Animals. C57BL/6 female mice (n=48, 9-10/group) were purchased from Charles River Breeding Labs, Wilmington, MA, USA) at 8-9 weeks of age. Half of the cohort was shipped directly to the NSRL in New York for irradiation with carbon and iron ions; the other half was shipped directly to Loma Linda for exposure to γ-rays and protons. Sham-irradiated controls were divided equally between the two Institutions. The animals were acclimatized at each facility for 1-2 weeks. Irradiations were coordinated between investigators at the two facilities so that exposures were performed at similar time-points. The mice irradiated at NSRL were shipped overnight within days post-exposure to LLU for subsequent housing. At 103 to 120 days after irradiation (hereafter referred to as day 110), the mice were rapidly euthanized in 100% CO_2 (27) for *in vitro* analyses. This study was approved by the appropriate Institutional Animal Care and Use Committees.

Whole-body irradiation. Different groups of unanesthetized mice received whole-body irradiation using 60-Cobalt (^{60}Co) γ-rays (1.17 and 1.33 MeV), LET=0.267 KeV/μm), protons ($^{1}H^{1+}$, 237 MeV/nucleon, dose averaged LET=0.4 KeV/μm, at the front of the target), carbon ($^{12}C^{6+}$, 292 MeV/nucleon, LET=12.9 KeV/μm), or iron ($^{56}Fe2^{6+}$, 964 MeV/nucleon, LET=151.5 KeV/μm). Immediately prior to irradiation, the animals were placed individually into rectangular plastic aerated boxes (30 mm x 30 mm x 60 mm). Identical procedures were implemented with control mice that received no radiation. Low-LET exposures (^{60}Co and protons) were performed in the Department of Radiation Medicine at Loma Linda University Medical Center (LLUMC), USA, as previously described (28-30). For ^{60}Co irradiation, a vertical beam from a retired AECL (Atomic Energy of Canada, Ltd., Commercial Products Division, Ottawa, Canada) Eldorado therapy unit was used to deliver the beam from the top (dorsally). Protons were delivered dorsally in 0.3 sec pulses every 2.2 sec. The dose was evaluated by placing a PTW Markus type ion chamber at the same target location as the animal. Three measurements were made, one was at the surface; in the next measurement, polystyrene (1.045 g/cm^3) was placed in front of the chamber to simulate the depth of half the animal and the final had polystyrene that simulated the full thickness of the animal. Less than 2% variability was found from surface dose to full depth. High-LET exposures (^{12}C and ^{56}Fe) were done at BNL at the NSRL in New York in July 2003; irradiations were done from the side (laterally) with a horizontal beam line according with standardized procedures. Measurements were made at the target site with an EG&G ORTEC 1 cm^3 ion chamber calibrated against a series of parallel plate and segment parallel plate ion chambers. ^{12}C was delivered in ~0.4 sec spills every ~2.0 sec and maximum possible LET drop off from front to back of the irradiation chamber was predicted to be ~4.6%. ^{56}Fe was delivered in ~0.4 sec spills every ~5.5 sec and drop off from front to back of the irradiation chamber was ~1.3%. All radiations were delivered in a single fraction of 2 Gy at dose rates ranging from 0.6 to 1.2 Gy/min. Particle radiations (protons, carbon and iron) were delivered at the entrance plateau region of the beam. Four to 8 mice were irradiated simultaneously.

Automated leukocyte analysis. Whole blood was collected by cardiac puncture in [K_2]EDTA-containing syringes at the time of euthanasia and analyzed with an ABC Vet Hematology Analyzer (Heska Corp., Waukesha, WI, USA). The analyzer provides white blood cell (WBC), lymphocyte, monocyte, granulocyte, red blood cell (RBC), and platelet (PLT) counts, hemoglobin (HGB) concentration, 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). The analyzer was also used to obtain WBC counts and three-part differentials in spleens that had been processed into single-celled suspensions; RBCs were lysed in 2 ml of cold lysing buffer.

Flow cytometry analysis of lymphocytes. Blood and spleen lymphocytes were evaluated using a FACSCalibur™ flow cytometer (Becton Dickinson, Inc., San Jose, CA, USA), 4-color mixtures of fluorescence-labeled monoclonal antibodies (mAb) (Pharmingen, San Diego, CA, USA) and conventional direct-staining techniques.

Lymphocytes were identified on the basis of cluster differentiation (CD) molecule CD45 (clone 30-F11) expression and side scatter. T, Th, Tc, B and natural killer (NK) cells were identified using mAb against CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), CD19 (ID3) and NK1.1 (PK136), respectively. In addition, T and B lymphocytes expressing a marker associated with memory cells, *i.e.,* CD62L (mel-14), were identified separately and in concert with mAb against the CD3 and CD19 markers. The 5,000–10,000 events acquired per tube were analyzed using CellQuest™ software version 3.1 (Becton Dickinson). The number of cells comprising each specific phenotype was based on the results from the automated hematology analyzer.

Mitogen-induced blastogenesis. Splenic leukocytes were dispensed into microtiter plates ($2x10^5$ cells/0.1 ml/well) followed by addition of 0.1 ml of medium containing phytohemagglutinin (PHA), concanavalin A (ConA), lipopolysaccharide (LPS) or medium with no mitogen, as previously described (22). All mitogens were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and pre-titrated for maximal response. The cells were pulse-labeled with ^{3}H-thymidine (^{3}H-TdR; specific activity=46 Ci/μmol; ICN Biochemicals, Costa Mesa, CA, USA) with1 μCi/50 μl/well) during the last 4 h of a 48 h incubation period. Cells were harvested and the radioactivity incorporated into cell DNA was quantified.

Relative organ masses. Mice were weighed at the time of euthanasia and spleen, thymus, lung and liver were excised. Organ masses relative to body mass were calculated for each animal: relative organ mass=organ mass (mg)/body mass (g).

Statistical analysis. The study was performed in two experiments. Half of the non-irradiated control mice were euthanized at the same time as the groups exposed to γ-rays and protons, whereas the other half was euthanized along with the carbon and iron irradiated animals. To minimize any day-to-day variability between experiments, values were normalized to the average of their respective 0 Gy controls for statistical analyses using the following equation [(Data Point from Specific Day / Mean of Controls from Specific Day) x Mean of All Controls]. The data were evaluated with one-way analysis of variance (ANOVA) using group as the independent variable. When indicated, Tukey's pairwise multiple comparison test was used to determine significant difference between sets comprised of two groups each. These analyses were performed using SigmaStat™ software, version 2.03 (SPSS Inc., Chicago, IL, USA). A *p*-value of <0.05 was selected to indicate significance.

Results

Leukocyte counts and three-part differential in blood and spleen. WBC counts and three-part differentials are presented in Figure 1. In the blood, low monocyte counts were noted in the groups exposed to protons and carbon ions compared to 0 Gy ($p<0.05$). In the spleen, all major leukocyte populations, except granulocytes, were elevated after γ-irradiation compared to controls ($p<0.05$). Lymphocyte and monocyte/macrophage counts were also higher in the Gamma group compared to the Iron group ($p<0.05$). Granulocyte counts were lower in both high-LET groups compared to both low-LET groups ($p<0.005$), but not 0 Gy controls.

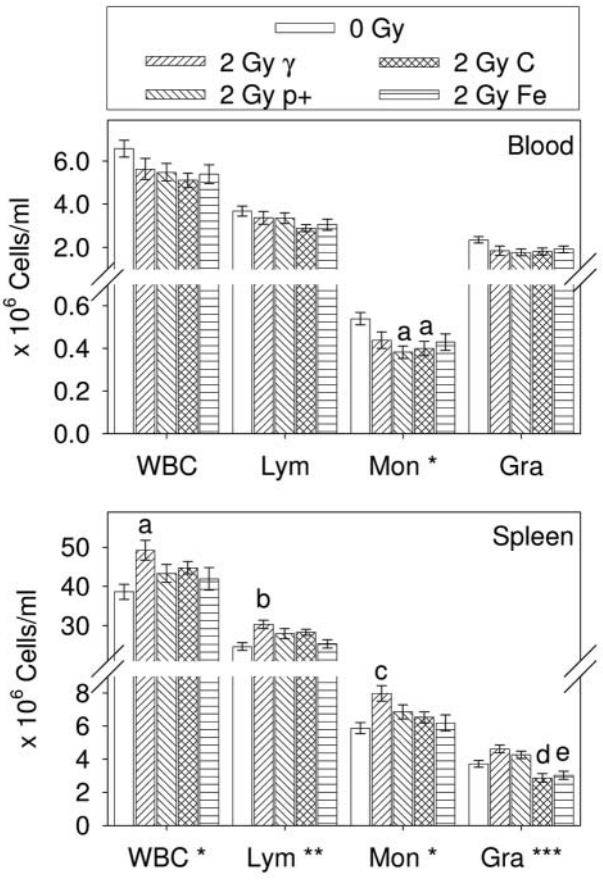

Figure 1. *Major leukocyte populations. Data were obtained using an automated hematology analyzer. Means±SEM are shown for 9-10 mice/group. One-way ANOVA: *p<0.05, **p<0.005 and ***p<0.001 for main effect of group. Tukeys: [a]p<0.05 vs. 0 Gy; [b]p<0.005 vs. 0 Gy, p<0.05 vs. Iron; [c]p<0.01 vs. 0 Gy, p<0.05 vs. Iron; [d]p<0.001 vs. Gamma and Protons; [e]p<0.001 vs. Gamma and p<0.005 vs. Protons.*

Lymphocyte populations and subpopulations. The data for T, B and NK cells are presented in Figure 2. In the blood, NK cells were the only major lymphocyte type affected by radiation; significantly low numbers were found in all irradiated groups compared to the 0 Gy Controls ($p<0.05$). In the spleen, T cell counts were high in the Carbon group compared to 0 Gy, whereas the Iron group had low counts compared to the Gamma and Carbon groups ($p<0.05$). Splenic B cells were elevated only in the Gamma-irradiated group compared to controls ($p<0.005$). NK cells were low in mice exposed to protons or carbon ions *vs.* 0 Gy ($p<0.05$).

There were no significant differences in circulating Th and Tc subsets for any group (Figure 3). In the spleen, Th cells were increased in the Carbon group *vs.* 0 Gy and

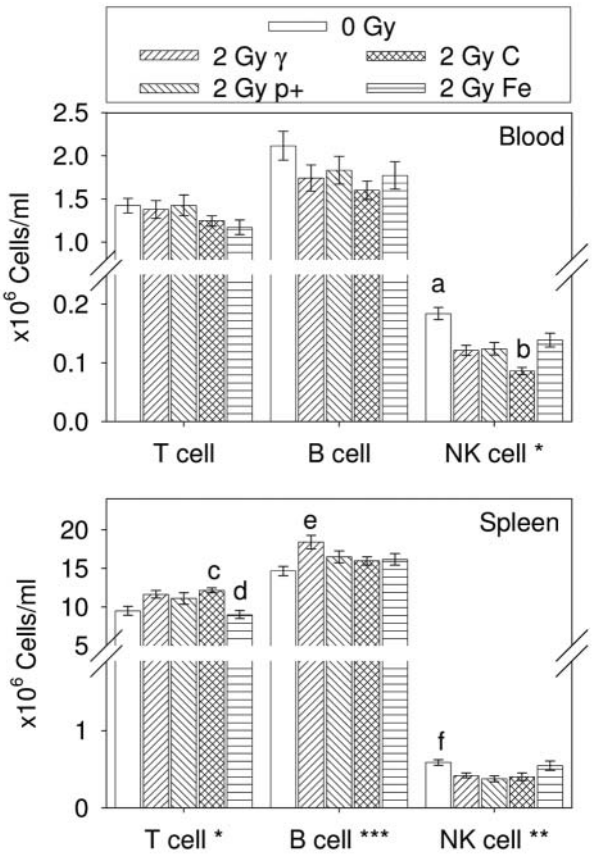

Figure 2. *Major lymphocyte populations. Data were obtained using fluorescence-labeled antibodies and flow cytometry. Means±SEM are shown for 9-10 mice/group. One-way ANOVA: *p<0.001, **p<0.005 and ***p<0.05 for main effect of group. Tukeys: [a]p<0.001 vs. Gamma, Protons, and Carbon, p<0.05 vs. Iron; [b]p<0.05 vs. Protons and Iron; [c]p<0.05 vs. Gamma and p<0.005 vs. Iron; [d]p<0.05 vs. Gamma; [e]p<0.05 vs. 0 Gy Controls; [f]p<0.01 vs. Protons and p<0.05 vs. Carbon.*

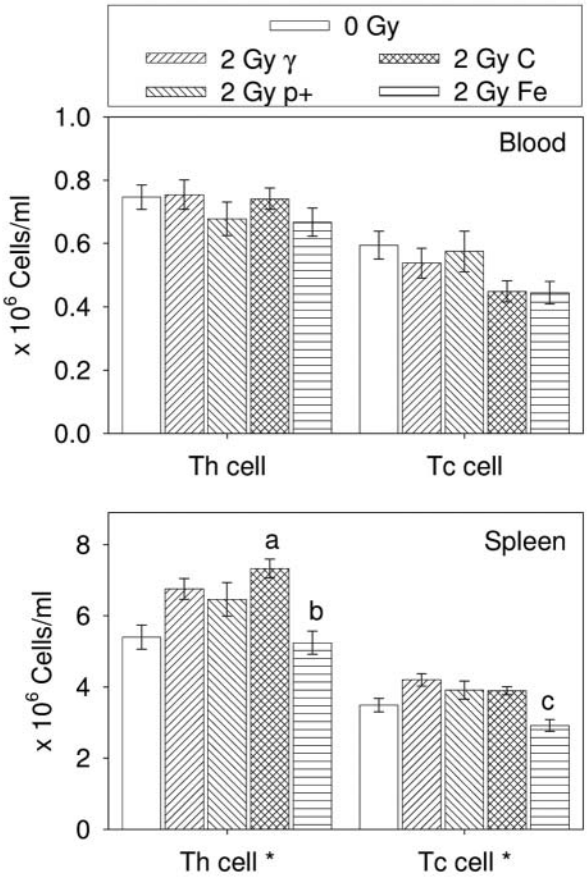

Figure 3. *T lymphocyte subpopulations. Data were obtained using fluorescence-labeled antibodies and flow cytometry. Means±SEM are shown for 9-10 mice/group. One-way ANOVA: *p<0.001 for main effect of group. Tukeys: [a]p<0.005 vs. 0 Gy Control; [b]p<0.05 vs. Gamma and p<0.005 vs. Carbon; [c]p<0.001 vs. Gamma, p<0.005 vs. Protons, and p<0.01 vs. Carbon.*

Iron ($p<0.005$). The Iron group was also lower than the γ-irradiated group ($p<0.05$). Tc counts were high in the Gamma, Proton and Carbon groups when compared to the Iron group ($p<0.01$), but no group varied significantly from 0 Gy. Figure 4 shows that the Th:Tc (CD4:CD8) ratio was elevated in the blood from the Carbon group compared to all other groups ($p<0.05$), and in the Iron group compared to 0 Gy ($p<0.05$). In the spleen, both high-LET groups had increased ratios compared to 0 Gy and Gamma groups ($p<0.05$). The Carbon group also had higher values than the Proton group ($p<0.01$).

In the blood, there were no significant differences between $CD62L^+$ groups for either T or B cells (data not shown). However, in the spleen, $CD62L^+$ T cells were significantly increased in the Gamma, Proton, and Carbon groups compared to animals receiving either 0 Gy or Iron ($p<0.05$, Figure 5). Splenic B cells with the marker increased only in the Gamma group when compared to the iron-irradiated mice ($p<0.05$).

Mitogen-induced blastogenesis. There were no significant differences among groups in DNA synthesis when splenocytes were stimulated by mitogens. The range of cpm values for each mitogen were as follows. PHA: 229,745±23,433 (Carbon) to 209,905±4,757 (Iron); ConA: 223,399±56,318 (Proton) to 155,617±10,144 (Iron); and LPS: 251,273±8,454 (Carbon) to 213,273±9,000 (Gamma).

Erythrocyte and platelet characteristics. Selected characteristics of circulating RBCs are reported in Table I. Iron ion radiation reduced RBC counts, hemoglobin and hematocrit compared to the 0 Gy and Gamma groups ($p<0.05$). The volume per RBC was increased in the Gamma group compared to 0 Gy and both high-LET groups ($p<0.05$).

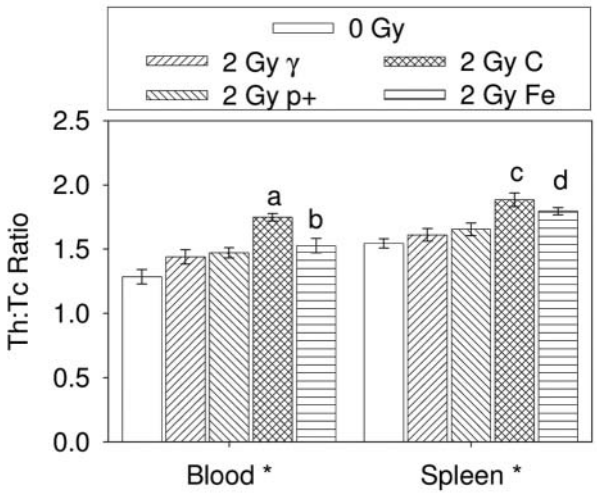

Figure 4. *T helper (Th): T cytotoxic (Tc) cell ratio. Data were obtained using fluorescence-labeled antibodies and flow cytometry. Means±SEM are shown for 9-10 mice/group. One-way ANOVA: *p<0.001 for main effect of group. Tukeys: [a]p<0.001 vs. 0 Gy Controls and Gamma, p<0.01 vs. Protons, and p<0.05 vs. Iron; [b]p<0.05 vs. 0 Gy Controls; [c]p<0.001 vs. 0 Gy Controls and Gamma, p<0.01 vs. Protons; [d]p<0.005 vs. 0 Gy Controls and p<0,05 vs. Gamma.*

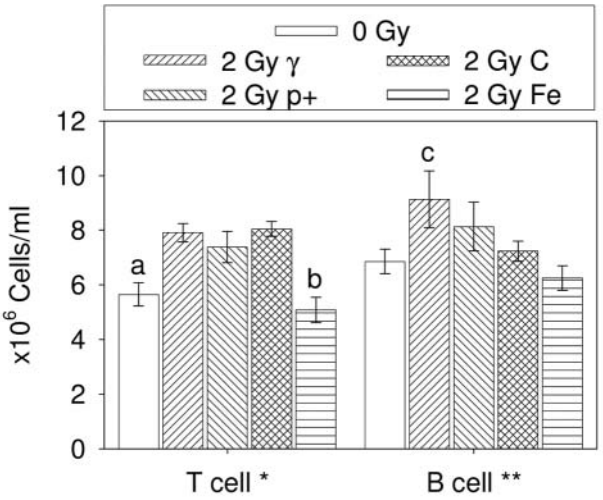

Figure 5. *Splenic T and B lymphocytes expressing CD62L. Data were obtained using fluorescence-labeled antibodies and flow cytometry. Means±SEM are shown for 9-10 mice/group. One-way ANOVA: *p<0.001 and **p<0.05 for main effect of group. Tukeys: [a]p<0.005 vs. Gamma and Carbon, p<0.05 vs. Protons; [b]p<0.001 vs. Gamma and Carbon, p<0.005 vs. Proton; [c]p<0.05 vs. Gamma.*

Table I. *Circulating red blood cell (RBC) parameters. Data were obtained using an automated hematology analyzer. Means±SEM are shown for 9-10 mice/group.*

	Controls	Gamma	Proton	Carbon	Iron
RBC (x10^9 cells/ml) *	8.8±0.2	9.1±0.3	8.6±0.2	8.3±0.2	7.5±0.4[a]
HGB (g/dl)**	12.7±0.2	12.8±0.2	12.2±0.2	12.1±0.2	11.8±0.2[b]
HCT (%)***	39.8±1.1	42.2±1.3	39.2±1.3	37.2±1.0	33.7±1.8[c]
MCV (μm^3)**	45.1±0.2	46.0±0.3[d]	45.3±0.2	45.0±0.2	44.5±0.3
MCH (pg)	14.5±0.2	14.1±0.3	14.3±0.2	14.7±0.1	14.6±0.1
MCHC (g/dl) *	32.2±0.5	30.5±0.8[e]	31.3±0.6	32.7±0.3	32.8±0.3
RDW (%) **	15.7±0.1	15.1±0.2	15.2±0.2	16.0±0.1[f]	15.7±0.2
PLT (x10^6 cells/ml)	935.0±41.0	853±42.0	797.0±32.0	851.0±36.0	873.0±47.0
MPV (μm^3)	9.5±0.1	9.1±0.2	9.3±0.2	9.7±0.2	9.2±0.2

One-way ANOVA: *$p<0.05$, **$p<0.005$ and ***$p<0.001$ for main effect of group. Tukeys: [a]$p<0.01$ *vs.* Controls and $p<0.001$ *vs.* Gamma; [b]$p<0.05$ *vs.* Controls and Gamma; [c]$p<0.05$ *vs.* Control and Carbon, $p<0.001$ *vs.* Gamma; [d]$p<0.001$ *vs.* Gamma, $p<0.05$ *vs.* Carbon; [e]$p<0.05$ *vs.* Carbon and Iron; [f]$p<0.005$ *vs.* Gamma and $p<0.05$ *vs.* Protons.

There were no significant differences in MCH. Although one-way ANOVA indicated a main effect of group for MCHC (hemoglobin concentration per RBC), there were no significant differences from controls in post-hoc analyses. However, the high-LET groups both had higher MCHCs than the γ-irradiated group. RDW was significantly higher in the Carbon group than in either the Gamma or Proton groups ($p<0.05$). Platelet counts and volume were similar among groups.

Body and organ masses. Although all exposed groups weighed slightly more than the non-irradiated controls, radiation had no significant effect on body mass. Mean values ranged from 25.2±0.4 g (0 Gy Controls) to 26.7±0.7 g (Iron). Spleen, thymus, and lung masses were also similar among groups (data not shown). Relative spleen mass (mg/g) ranged from 3.9±0.1 (0 Gy Controls) to 4.1±0.2 (Iron); relative thymus mass (mg/g) ranged from 2.9±0.2 (Protons) to 3.3±0.2 (0 Gy Controls); and relative lung mass (mg/g) ranged from 6.6±0.3 (Protons) to 7.3±0.4 (Iron). In contrast, radiation had a main effect on both liver mass ($p<0.001$) and relative liver mass ($p<0.05$). For liver mass, *post hoc* analysis showed that both the Carbon- and the Iron-irradiated mice had significant hepatomegaly compared to 0

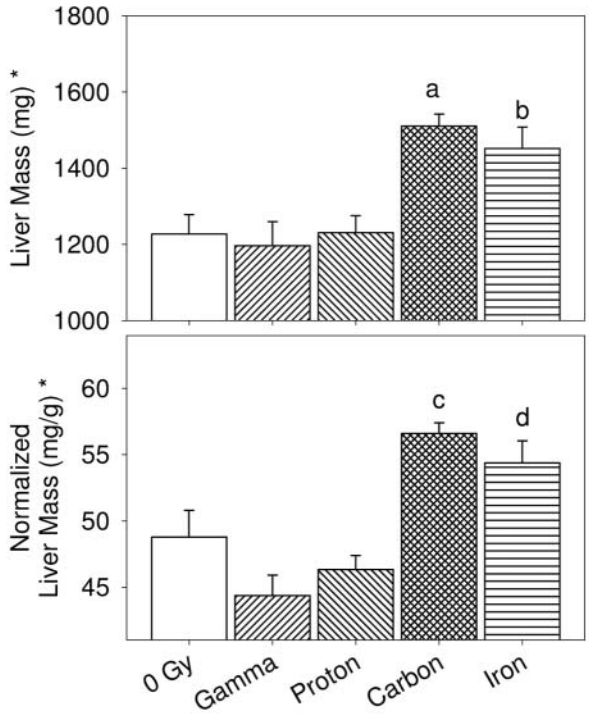

Figure 6. *Liver mass. Means±SEM are shown for 9-10 mice/group. One-way ANOVA: *p<0.001 for main effect of group. Tukeys: [a]p<0.005 vs. 0 Gy and Protons, p<0.001 vs. Gamma; [b]p<0.05 vs. 0 Gy and Protons, p<0.01 vs. Gamma; [c]p<0.01 vs. 0 Gy and p<0.001 vs. Gamma and Protons; [d]p<0.001 vs. Gamma and p<0.005 vs. Protons.*

Gy Controls and low LET groups ($p<0.05$, Figure 6). Post-hoc analysis of relative liver mass showed that the Carbon group had hepatomegaly compared to 0 Gy Controls ($p<0.01$). However, the means for both high-LET groups were elevated compared to both low-LET groups ($p<0.005$).

Discussion

In the present study, WBC counts in the blood were similar among all groups, suggesting that hematopoietic processes in the bone marrow that continuously replenish immune and erythropoietic systems were intact. However, under conditions of stress, compensatory mechanisms in organs such as the spleen and liver are activated. In contrast to the blood, total WBC, lymphocyte and monocyte/macrophage counts were elevated in spleens from the γ-irradiated group. Granulocytes were greater in both low-LET-irradiated groups compared to both high-LET-irradiated groups. These findings suggest that exposure to the low-LET radiations resulted in a more robust regeneration of leukocytes. It also seems possible that the increased leukocyte numbers may be a response to dying cells that were sub-lethally damaged at the time of irradiation.

Differences dependent upon radiation quality and/or body compartment were also found in most leukocyte populations. Examples include: a) a decrease in blood monocytes in the Proton and Carbon groups, with an increase in splenic monocytes in the Gamma group; b) an increase in splenic, but not circulating, T lymphocytes in the Carbon group; c) elevated CD4:CD8 T cell ratio in both high-LET groups in blood and spleen; d) an increase in splenic, but not circulating, B cell counts in the γ-irradiated group; and e) a decrease in NK cells in both compartments for the Proton and Carbon groups, but only in the blood for the Gamma and Iron groups. Although the data reported here are generally in agreement with our previous findings with low- and high-LET forms of radiation, there was one inconsistency. Here we found no difference in circulating B cell numbers. However, in the previous study with 2 Gy ^{56}Fe, we found an increase in this parameter (22). There is no obvious explanation for this apparent discrepancy at this time. Nevertheless, the collective findings clearly demonstrate abnormalities in the numbers and proportional balance of the quantified populations.

To our knowledge, this is the first comparison of CD62L expression by T and B cells after *in vivo* exposure to the radiations used here. The CD62L molecule is associated with memory lymphocytes that migrate rapidly and proliferate extensively upon antigen stimulation (31). CD62L (L-selectin), a member of the selectin adhesion molecule family, binds to determinants on high endothelial venules in peripheral lymph nodes, and thus facilitates cell migration to injured sites. Although $CD62L^+$ T or B cell counts in the blood were similar among groups, splenic $CD62L^+$ T counts were high in all irradiated animals except those exposed to iron ions. An increase in splenic $CD62L^+$ T cells, coincident with an increase in macrophages (as demonstrated in the present study), would be consistent with continuing efforts to repair radiation-induced tissue damage and regain homeostasis.

Erythrocyte counts, hemoglobin and hematocrit were low only in the mice exposed to iron ions, the radiation with the highest LET. Although the mice received a larger dose of ^{56}Fe radiation than would be expected even during extended space missions, radiation combined with other factors may exacerbate the anemia commonly noted in astronauts (32, 33). The findings imply that kidney function was impaired, since this organ is the major source of erythropoietin, a hormone that regulates RBC production. Alternatively, iron ion irradiation may have caused greater damage to lineage-committed erythroid progenitors in the bone marrow than the other forms of radiation. Differences in the microenvironment, *i.e.*, discrete oxic and hypoxic regions, as well as areas with gradients of oxygenation, may also be a factor (34, 35).

The data show that radiation had no significant effect on body mass at the time of measurement, suggesting that whole-body exposure to a relatively high dose of either low- or high-LET radiation has little or no long-term influence on this parameter. The weight loss commonly seen in astronauts has been associated with multiple factors including low food intake, bone decalcification, muscle atrophy, cardiovascular degeneration, and decreased plasma volume (36). We and others have also noted low body mass in rodents when measured after the return from spaceflight missions (37-39). However, in ground-based studies, decreased body mass shortly after radiation exposure is a well-known phenomenon (28, 40), possibly due to alterations in lipids and fatty acids that may collectively result in metabolic changes that affect the whole organism (41-45). Our data suggests this decrease in body mass is transient.

Hepatomegaly was seen after carbon ion irradiation. While the liver/body mass ratio is usually tightly regulated (46, 47), others have noted long term liver hypertrophy following clinical treatment with heavy charged particles, where the degree of enlargement increased with increasing volume exposed (48).

The long-term effect of radiation on the liver could be due to its vasculature architecture, with structurally defined functional subunits (FSU) along each vascular branch. Because cells from each FSU do not typically repopulate adjacent FSUs, this architecture tends to result in an enhanced radiosensitivity (49). Changes in liver mass could also be explained by activation of the sympathetic nervous system or hypothalamus-pituitary-adrenal axis (47). However, all groups were handled similarly and it is unlikely that a significant increase seen only in the Carbon group could be attributed to a stress response. Furthermore, there were no significant changes in stress sensitive spleen and thymus masses across groups.

Another possible explanation is an increase in cell proliferation. Partial hepatectomy and other forms of liver injury induce regeneration, involving bone marrow-derived cells that give rise to hepatocytes, as well as other cell types residing in the liver (50). There appear to be no published reports for this process following damage due to high-LET radiation. However, should this be the case, there is evidence that enhanced cell proliferation increases risk for malignant transformation. A recent study comparing the effects of whole-body exposure to X-rays and carbon ions found that the life span of carbon-irradiated mice was reduced after 1.6 Gy (0.4 Gy delivered for 4 consecutive weeks), with liver tumors being a prominent cause of death (51). Finally, the increased liver mass may reflect the clearance of system-wide damage, although this would likely be minimal at nearly 4 months post-exposure.

Our previous studies have shown that splenic and thymic atrophy is profound during the first week after exposure to γ-rays, protons, and iron ions (22, 28, 40). However, we have also found that the functional status and interactive potential of the cells may be drastically altered when challenged with immunogenic components of infectious agents (52, 53). The lack of differences in spleen and thymus masses among groups at 4 months indicates that organ regeneration was complete in the global sense, regardless of radiation quality.

In summary, the data show that whole-body irradiation of mice produced significant changes in leukocyte and erythrocyte populations that are important in maintenance of good health. The aberrations were noted almost 4 months after radiation exposure, *i.e.,* equivalent to nearly one-fifth of the lifetime of a mouse. In human terms, this time interval would be roughly equivalent to 16 years. In some cases, significant differences in cell populations were associated with radiation quality, but the body compartment evaluated was an important factor in the results. Overall, differences among groups were strikingly more pronounced in the spleen than in the blood. The changes noted in the liver suggest that the effect of LET may be dependent on structural organization of the tissue.

Rather than the traditional dose response or time course studies, we chose to keep dose and time constant while varying LET. Now that we have established that the effect of radiation on our parameters depends on LET, a closer examination of the dose response curves of each radiation type is necessary. This will allow us to determine if the changes noted are due to shifts in the overall biological effectiveness of each curve, or due to LET-dependent shifts in latency. Clearly, additional investigation is required to identify specific mechanisms and to determine the seriousness of the abnormal findings.

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