Total-body irradiation with high-LET particles: acute and chronic effects on the immune system

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Gridley, Daila S., Michael J. Pecaut, and Gregory A. Nelson. Total-body irradiation with high-LET particles: acute and chronic effects on the immune system. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R677–R688, 2002; 10.1152/ajpregu.00435.2001.—Although the immune system is highly susceptible to radiation-induced damage, consequences of high linear energy transfer (LET) radiation remain unclear. This study evaluated the effects of 0.1 gray (Gy), 0.5 Gy, and 2.0 Gy iron ion ($^{56}Fe^{26}$) radiation on lymphoid cells and organs of C57BL/6 mice on *days 4* and *113* after whole body exposure; a group irradiated with 2.0 Gy silicon ions (^{28}Si) was euthanized on *day 113*. On *day 4* after ^{56}Fe irradiation, dose-dependent decreases were noted in spleen and thymus masses and all major leukocyte populations in blood and spleen. The $CD19^+$ B lymphocytes were most radiosensitive and $NK1.1^+$ natural killer (NK) cells were most resistant. $CD3^+$ T cells were moderately radiosensitive and a greater loss of $CD3^+/CD8^+$ T_C cells than $CD3^+/CD4^+$ T_H cells was noted. Basal DNA synthesis was elevated on *day 4*, but response to mitogens and secretion of interleukin-2 and tumor necrosis factor-α were unaffected. Signs of anemia were noted. By *day 113*, high B cell numbers and low T_C cell and monocyte percents were found in the 2.0 Gy ^{56}Fe group; the 2.0 Gy ^{28}Si mice had low NK cells, decreased basal DNA synthesis, and a somewhat increased response to two mitogens. Collectively, the data show that lymphoid cells and tissues are markedly affected by high linear energy transfer (LET) radiation at relatively low doses, that some aberrations persist long after exposure, and that different consequences may be induced by various densely ionizing particles. Thus simultaneous exposure to multiple radiation sources could lead to a broader spectrum of immune dysfunction than currently anticipated.

iron ion radiation; silicon ion radiation; lymphocytes; immunomodulation

THIS IS ONE OF A SERIES OF studies evaluating immunological status after exposure to different forms of radiation. The underlying impetus for these investigations is the lack of sufficient data to establish realistic health risk estimates for astronauts on extended voyages in space. There is an increasing sense of urgency to obtain the needed data, given the fact that residency on the International Space Station has become a reality and plans for manned missions to the moon and Mars are in progress. Although different aspects of the space environment can influence many physiological systems (3, 25, 39, 46), immune dysfunction due to radiation exposure, with its potentially serious consequences, is a paramount concern (4, 43). Furthermore, the combination of radiation, weightlessness, and psychological stress may exert additive or synergistic effects that severely compromise resistance to infections and other diseases (49).

During a 2- to 3-yr Mars mission, the total radiation dose that astronauts may receive could reach 3.0 gray (Gy) (12, 35, 43, 45). The radiation quality is likely to include a variety of high-energy and high-charge ions particles >100 MeV/nucleon with high-linear energy transfer (LET). These particles are densely ionizing and deposit >50% of their energy along their linear tracks (31). Iron ions ($^{56}Fe^{26+}$) have received much attention, because they are the most densely ionizing particles present in relatively large amounts in galactic cosmic rays. Furthermore, it has been estimated that 3% of the cells within one individual will be traversed on average by one iron ion during a 3-yr mission (7). Although protons account for approximately 85–90% of deep space radiation, high-LET particles (which represent only ~1% of space radiation) may have substantially greater biological effects (12, 43). Reports from more than three decades ago indicate that radiations with high LET tend to produce exponential cell inactivation curves (2, 48). More recently, it has been demonstrated that ^{56}Fe and other forms of high-LET radiation are more effective than low-LET photons (i.e., γ-rays) in depressing enzymatic repair mechanisms, decreasing variations in cell radiosensitivity, minimizing protective effects of neighboring cells in organized tissues, increasing chromosomal aberrations, and inducing neoplastic transformation of cells (1, 19, 32, 47, 50, 53, 54).

Recent ground-based studies, utilizing total doses within the range expected during extended space residence, have shown that ionizing radiation can profoundly influence many aspects of the immune system (17, 18, 21, 24, 26, 34, 44). However, the great majority of these studies have been performed with γ-rays or

X-rays, forms of radiation generally most available for research. Data regarding changes in immune system structure and function after total-body exposure to high-LET radiation are very sparse. In previous studies, we have reported on the acute effects of total-body irradiation with γ-rays (^{60}Co) and protons in the C57BL/6 mouse strain (14, 15, 27–29, 36, 38). The present study expands these investigations of lymphoid organs and bone marrow-derived cells to high-LET radiation in the same animal model.

METHODS

Animals and whole body irradiation. Female C57BL/6 mice ($n = 136$) were purchased from Charles River Breeding Laboratories, Wilmington, MA, at 8–9 wk of age, shipped directly to Brookhaven National Laboratory, and acclimatized for 1 wk under standard vivarium conditions. Immediately before exposure using the Alternative Gradient Synchrotron, the mice were placed into a vertical stack of four ventilated $3 \times 3 \times 6$-cm boxes composed of polystyrene (tissue equivalent, hence attenuation of the beam was minimal). The mice were not anesthetized, but movement was limited to comfortable breathing due to the relatively small box size. Two additional same size boxes, each containing a phantom (i.e., polystyrene cylinders with conical ends) were placed above and below the boxes containing the animals for support in the holder apparatus (Fig. 1). Sham-irradiated controls were placed into the same size boxes for the same length of time as the animals receiving the maximum radiation dose. Lead foils were placed in the beam line to obtain a circular 7.5-cm beam during ^{56}Fe and ^{28}Si irradiation and the intensity profile was tuned to a larger elliptical area so that uniformity of the beam across mouse bodies was maximized. The delivered dose was intermittently quantified using a thermal luminescent dosimeter array placed on one of the boxes containing a phantom. A portion of the animals ($n = 90$; 45 mice for euthanasia on *day 4* and 45 mice for euthanasia on *day 113*) were irradiated with heavy iron ions (^{56}Fe, Z = 26, 1087 MeV/nucleon at extraction and 1055 MeV/nucleon after passage through the vacuum line plus 2

meters of air, LET = 148.2 keV/μm at target). Total doses delivered were 0.1 Gy, 0.5 Gy, and 2.0 Gy. An additional group ($n = 14$ mice) was irradiated with 2.0 Gy silicon ions (^{28}Si, Z = 14, 1200 MeV/nucleon at extraction and 1,182 MeV/nucleon after passage through the vacuum line and 2 meters of air, LET = 42.1 keV/μm at target). The ^{56}Fe and ^{28}Si irradiations were performed at the entrance plateau region of the beam; each dose was delivered in a single fraction with ±10% uniformity and a dose rate of ~1 Gy/min. The contribution of secondary particles at the center of the mouse would be similar to what has been previously described for tissue culture flasks with water (55, 56). A maximum of four mice were irradiated per exposure. A portion of the ^{56}Fe-irradiated animals were weighed and euthanized at 4 days postexposure by rapid CO_2 asphyxiation at Brookhaven National Laboratory, whereas the remaining mice (14–16 mice/each dose of ^{56}Fe plus 14 ^{28}Si-irradiated mice plus 17 nonirradiated controls) were shipped to Loma Linda University Medical Center and euthanized for assay on *days 109-114* (hereafter referred to as *day 113*). After irradiation, animals were observed daily for signs of toxicity. In addition, the long-term animals underwent behavioral assessment (unpublished observations). The irradiations and subsequent immunological assessments were performed in two identical experiments at each time point and the data from each group were appropriately pooled. This study was approved by the Loma Linda University and Brookhaven National Laboratory Animal Care and Use Committees.

Body and organ masses and blood and spleen collection. Spleen and thymus masses were determined at the times of euthanasia and relative organ mass was calculated: relative organ mass = [organ mass (mg)]/body mass (g). Whole blood, collected in K_2 EDTA-containing syringes by cardiac puncture at the time of euthanasia, and single-celled suspensions of spleen leukocytes were obtained as described previously (13). The *day 4* postirradiation samples were shipped in sterile screw-capped vials on wet ice by courier to Loma Linda University for analyses, which were performed well within 24 h after euthanasia.

Fig. 1. Configuration of setup during mouse irradiation with heavy ions. Polystyrene boxes were stacked vertically and whole body irradiations were performed with a horizontal beam line (represented by the letter Z). The mice were exposed to the entrance plateau region of ^{56}Fe and ^{28}Si Bragg curves within a 7.5-cm circular beam. The intensity profile was tuned to a larger elliptical area so that uniformity of the beam across mouse bodies was maximized (±10%).
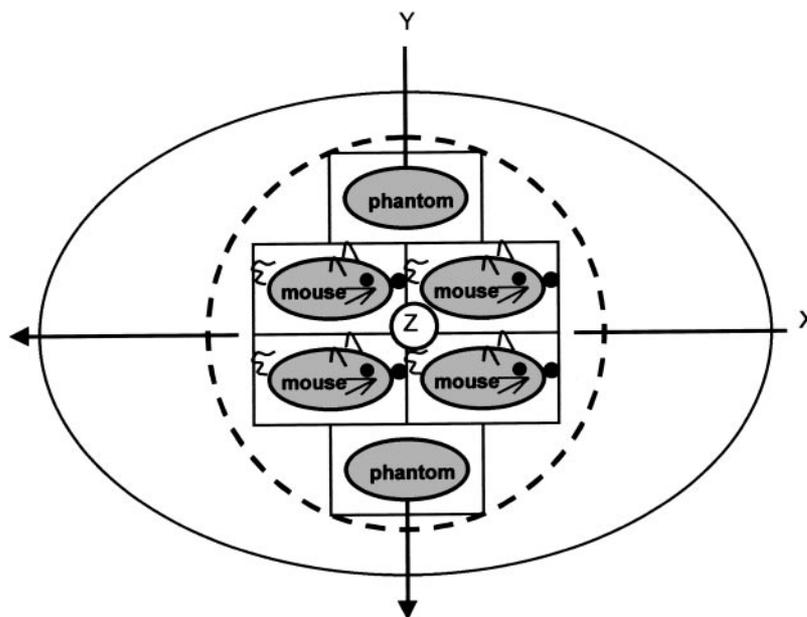
Analysis of blood and spleen with hematology analyzer. Whole blood samples (12 μl) were evaluated using the ABC Vet Hematology Analyzer (Heska, Waukesha, WI). White blood cell (WBC), monocyte, granulocyte, red blood cell (RBC), and thrombocyte counts, hemoglobin concentration, and hematocrit (percentage of whole blood composed of RBC) were determined. Some of these measurements were used to calculate the 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), and mean platelet volume (MPV). Only WBC, lymphocyte, granulocyte, and monocyte counts were obtained for the spleen after RBC lysis.

Flow cytometry analysis of lymphocyte and stem cell populations. Whole blood and spleen leukocyte samples were evaluated using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA), four-color mixtures of monoclonal antibodies (mAb) (Pharmingen, San Diego, CA), standard direct-staining techniques, and a "no lyse-no wash" procedure. The mAb were labeled with FITC, R-phycoerythrin, allophycocyanin, or peridinin chlorophyll protein and directed against $CD3^+$ mature T cells; $CD4^+$ T helper (T_H) cells; $CD8^+$ T cytotoxic (T_C) cells, $CD19^+$ B cells; and $NK1.1^+$ natural killer (NK) cells. Spleen samples from *day 113* mice were also analyzed for stem cells (based on size and side scatter) and for cells expressing CD34 and/or Ly-6. Lymphocytes were gated on $CD45^+$ cells and side scatter. The percentages of $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cells were based on the total lymphocyte population rather than on the total $CD3^+$ cells, because this has given more consistent and reproducible data using the "no lyse-no wash" procedure, as specified by the commercial source of the reagents. Analysis of at least 5,000 events per tube was performed using CellQuest software version 3.1 (Becton Dickinson). The number of cells for each population was calculated as follows: no. of cells in population/ml = no. of lymphocytes/ml × percentage of population.

Spontaneous and mitogen-induced blastogenesis. These assays were performed as previously described (13, 29). In quantification of basal proliferation, aliquots of whole blood and spleen leukocytes were diluted with supplemented RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) and dispensed into wells of microculture plates. One μCi of [³H]-thymidine ([³H]TdR; specific activity = 46 Ci/μmol; ICN Biochemicals, Costa Mesa, CA) was immediately added, and the plates were incubated for 3 h at 37°C. In addition, spleen leukocytes (2×10^5/0.1 ml medium/well) were dispensed into microtiter plates with phytohemagglutinin (PHA), concanavalin A (ConA), lipopolysaccharide (LPS), or no mitogen (2×10^5 cells/0.2 ml total volume/well; mitogens were from Sigma, St. Louis, MO). [³H]TdR (1 μCi·50 μl⁻¹·well⁻¹) was added for the final 4 h of a 48-h incubation period. In both assays, cells were harvested and the incorporated radioactivity was quantified. Spleen cell response to the mitogens was expressed as counts/min and as a stimulation index (SI): SI = (counts/min with mitogen − counts/min without mitogen)/ counts/min without mitogen.

Quantification of interleukin-2 and tumor necrosis factor-α in spleen cell supernatants. Before testing for interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α), spleen leukocytes were incubated with PHA for 48 h as described above but without [³H]TdR. Supernatants were aspirated, cells and debris were removed by centrifugation, and the levels of IL-2 and TNF-α were quantified using ELISA kits (Quantikine: R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The concentration of each cytokine in the test samples was interpolated from the appropriate standard curve.

Statistical analysis. Means and SE were obtained using one-way ANOVA, which also gave a *P* value for difference among groups. Tukey's pairwise multiple comparison test was used to determine significant difference among each set of two groups. The *P* values obtained with one-way ANOVA are presented in the text, whereas those with Tukey's test are shown in the tables and figures. Each irradiated group euthanized on *days 4* and *113* was compared with the respective 0 Gy control group euthanized at the same time point. Correlation among each of the measurements and radiation dose was determined using linear regression analysis; formulae and r^2 values are presented only for the measurements with $r^2 > 0.5$. These analyses were performed using SigmaStat software, version 2.03 (SPSS, Chicago, IL) and a *P* value of <0.05 was selected to indicate significance.

RESULTS

Body and lymphoid organ mass. Total body masses were similar among groups at both times of euthanasia. Means on *day 4* ranged from 19.2 ± 0.3 g (0.5 Gy) to 20.1 ± 0.2 g (0.1 Gy), whereas the *day 113* values ranged from 23.7 ± 0.3 g (0.1 Gy) to 24.64 ± 0.4 g (2.0 Gy ²⁸Si). However, on *day 4*, significant dose-dependent decreases in mass were noted for both the spleen and thymus (Fig. 2, $P < 0.005$). In post hoc analysis, only 2.0 Gy caused a significant decrease in spleen mass compared with 0 Gy and 0.1 Gy. Thymus mass after 2.0 Gy exposure was significantly decreased compared with all other groups, and the 0.5 Gy group had lower mass than the 0.1 Gy group. By *day 113* (Fig. 2), organ mass differences were no longer apparent.

Total leukocytes and major leukocyte populations. The data in Table 1 (blood) and Table 2 (spleen) show that on *day 4* highly significant radiation dose-dependent decreases existed in total WBC counts in both body compartments ($P < 0.001$). Tukey analysis of blood data indicated that 2.0 Gy caused a significant decrease in WBC and in all three major leukocyte populations compared with all other groups. With 0.5 Gy, a significant reduction was found only for WBC and lymphocyte numbers, whereas with 0.1 Gy only the lymphocytes were low. In the spleen (Table 2), a radiation dose effect was noted on *day 4* for WBC counts, number of lymphocytes, and granulocytes ($P < 0.001$) as well as percentages of granulocytes ($P < 0.05$). Pairwise comparisons showed highly significant reductions with 2 Gy in WBC, lymphocyte, and granulocyte numbers. Percentages of each cell population reflected the drop in each respective cell type as well as the degree of change in other cell types. In blood, lymphocyte percents were significantly decreased (0.5 Gy and 2.0 Gy groups), whereas percentages of granulocytes (2.0 Gy) and monocytes (0.5 Gy and 2.0 Gy groups) were increased. In spleen, granulocyte percents (2.0 Gy) were increased, but no other proportional changes reached statistical significance.

By *day 113*, there were significant radiation dose-dependent reductions in monocyte-macrophage percentages in both blood ($P < 0.001$) and spleen ($P < 0.05$) (Tables 1 and 2, respectively), whereas a dose-
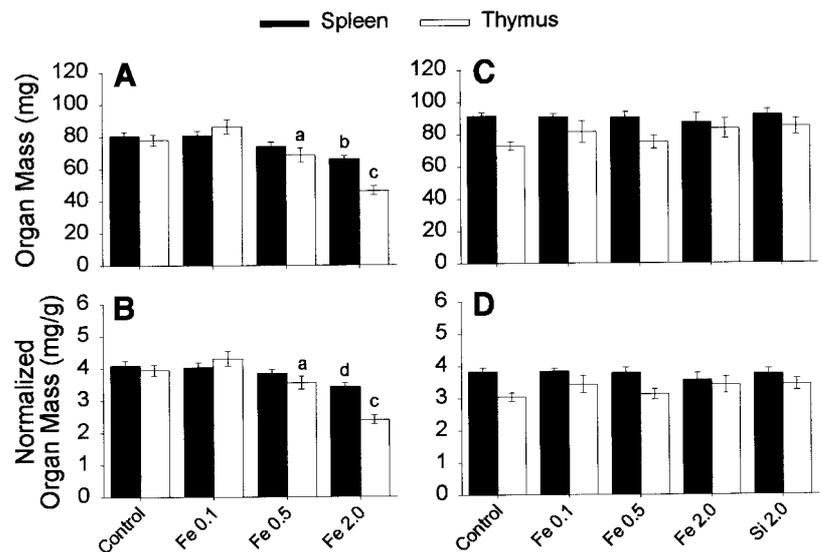
Fig. 2. Lymphoid organ mass. *A* and *C*: organ mass on *days 4* (*A*) and *113* (*C*); *B* and *D*: normalized organ mass on *days 4* (*B*) and *113* (*D*). Each bar represents the mean ± SE. [a]$P < 0.05$ vs. 0.1 Gy; [b]$P < 0.001$ vs. control and 0.1 Gy; [c]$P < 0.001$ vs. all other groups; [d]$P < 0.05$ vs. control and 0.1 Gy. Relative organ mass = [organ mass (mg)]/body mass (g).

dependent increase in lymphocyte numbers was present only in blood ($P < 0.05$). Post hoc analysis revealed that 2.0 Gy ^{56}Fe and 2.0 Gy ^{28}Si irradiation resulted in significantly lower monocyte percentages in blood compared with 0 Gy and 0.1 Gy ^{56}Fe; this occurred also in the spleen, but only in the group given 2.0 Gy ^{28}Si. Lymphocyte numbers were increased with 2.0 Gy ^{56}Fe compared with the 0 Gy group.

Lymphocyte populations. The data for CD3$^+$ T and CD19$^+$ B cells in blood and spleen are shown in Fig. 3. On *day 4*, the CD3$^+$ T cell counts were significantly dependent on radiation dose in both body compartments ($P < 0.001$). Post hoc analysis showed that the 2.0 Gy group had significantly lower values than all other groups. Similarly, CD19$^+$ B-cell counts decreased with dose in both compartments ($P < 0.001$) and the reduction in blood with 2.0 Gy was significant compared with all other groups; the 0.5 Gy dose caused a decrease compared with 0 Gy and 0.1 Gy. Splenic B cell numbers were lower in the 2.0 Gy group than in all other groups. With respect to proportional changes on *day 4*, a dose-dependent increase in CD3$^+$ T cell percents occurred in blood ($P < 0.001$), but not spleen. In contrast, CD19$^+$ B cell percents decreased with in-

creasing dose in both body compartments ($P < 0.001$). This dose response in the proportions of both T and B cells was linear ($r^2 > 0.7$).

On *day 113*, there was a dose-dependent effect on CD3$^+$ T cell percentages in the blood ($P < 0.05$), but not spleen, with the 2.0 Gy ^{56}Fe group having lower values than the 0 Gy and 0.5 Gy groups (Fig. 3). The numbers and percentages of CD19$^+$ B lymphocytes were enhanced in a dose-dependent manner only in blood ($P < 0.05$). Further analysis showed that after irradiating with 2.0 Gy ^{56}Fe, the circulating B cell counts and percents were elevated compared with 0 Gy.

Figure 4 shows the effect of radiation dose on CD3$^+$/CD4$^+$ T$_H$ and CD3$^+$/CD8$^+$ T$_C$ populations. On *day 4*, T$_H$ cell numbers in blood decreased with increasing dose ($P < 0.001$), and animals exposed to 2.0 Gy had significantly lower counts than all other groups. Although there were also dose-dependent decreases in the splenic T$_H$ population ($P < 0.001$), only the 2.0 Gy group had a lower mean than the 0 Gy and 0.5 Gy groups. T$_C$ cell counts in blood decreased significantly ($P < 0.001$), and those for the 2.0 Gy group were lower than all other groups. There was a similar dose-dependent decrease in splenic T$_C$ cell counts ($P < 0.001$), but

Table 1. *White blood cells and major leukocyte populations in peripheral blood*

Group	WBC	Lymphocytes	Granulocytes	Monocytes
Day 4				
0 Gy	4.87 ± 0.40[a]	$3.02 \pm 0.25 (63.0 \pm 1.1)$	$1.50 \pm 0.12 (28.9 \pm 1.1)$	$0.35 \pm 0.04 (7.7 \pm 0.3)$
0.1 Gy ^{56}Fe	4.70 ± 0.32	$2.98 \pm 0.22 (64.1 \pm 0.6)$	$1.39 \pm 0.09 (27.6 \pm 0.6)$	$0.33 \pm 0.03 (7.7 \pm 0.4)$
0.5 Gy ^{56}Fe	3.63 ± 0.31[b]	2.09 ± 0.16[b](59.9 ± 1.5)[e]	$1.25 \pm 0.12 (30.7 \pm 0.9)$	$0.29 \pm 0.03 (9.1 \pm 0.3)$
2.0 Gy ^{56}Fe	1.66 ± 0.07[c]	0.87 ± 0.03[c](56.0 ± 1.5)[f]	0.67 ± 0.04[c](34.4 ± 1.3)[f]	0.12 ± 0.01[c](9.2 ± 0.4)
Day 113				
0 Gy	2.97 ± 0.16	$1.93 \pm 0.10 (68.3 \pm 1.1)$	$0.79 \pm 0.06 (23.2 \pm 1.1)$	$0.19 \pm 0.01 (8.3 \pm 0.2)$
0.1 Gy ^{56}Fe	3.32 ± 0.26	$2.21 \pm 0.17 (68.0 \pm 1.2)$	$0.89 \pm 0.08 (23.5 \pm 1.1)$	$0.22 \pm 0.02 (8.2 \pm 0.3)$
0.5 Gy ^{56}Fe	3.48 ± 0.21	$2.34 \pm 0.18 (68.8 \pm 1.4)$	$0.91 \pm 0.09 (23.0 \pm 1.3)$	$0.24 \pm 0.02 (7.9 \pm 0.3)$
2.0 Gy ^{56}Fe	4.09 ± 0.44	2.75 ± 0.28[b](69.1 ± 1.0)	$1.08 \pm 0.14 (23.3 \pm 0.9)$	$0.26 \pm 0.03 (7.0 \pm 0.3)$[b]
2.0 Gy ^{28}Si	3.69 ± 0.37	$2.50 \pm 0.25 (69.1 \pm 1.1)$	$0.97 \pm 0.10 (23.5 \pm 1.1)$	$0.22 \pm 0.03 (6.9 \pm 0.3)$[b]

Values are mean ± SE; numbers in parentheses are the cell percentages. [a]Number of cells $\times 10^6$/ml; data obtained with the ABC Vet Hematology Analyzer. WBC, white blood cells. [b]$P < 0.05$ vs. 0 Gy; [c]$P < 0.001$ vs. all other groups; [d]$P < 0.01$ vs. 0 Gy and 0.1 Gy; [e]$P < 0.05$ vs. 0.1 Gy; [f]$P < 0.005$ vs. 0 Gy and 0.1 Gy; [g]$P < 0.05$ vs. 0 Gy and 0.1 Gy.

Table 2. *White blood cells and major leukocyte populations in the spleen*

Group	WBC	Lymphocytes	Granulocytes	Macrophages
Day 4				
0 Gy	30.75 ± 3.00^a	$20.95 \pm 1.84 (69.2 \pm 0.8)$	$7.83 \pm 0.97 (24.3 \pm 0.8)$	$0.58 \pm 0.19 (6.1 \pm 0.2)$
0.1 Gy ^{56}Fe	23.97 ± 3.14	$16.42 \pm 2.07 (69.6 \pm 0.8)$	$6.04 \pm 0.90 (23.9 \pm 0.8)$	$0.44 \pm 0.09 (6.0 \pm 0.1)$
0.5 Gy ^{56}Fe	25.52 ± 2.06	$17.73 \pm 1.32 (70.2 \pm 0.7)$	$6.13 \pm 0.60 (23.2 \pm 0.6)$	$0.52 \pm 0.08 (6.2 \pm 0.2)$
2.0 Gy ^{56}Fe	14.51 ± 1.08^b	$10.27 \pm 0.70 (71.9 \pm 0.5)$	$3.25 \pm 0.25^c (21.6 \pm 0.4)^d$	$0.55 \pm 0.07 (6.1 \pm 0.2)$
Day 113				
0 Gy	23.59 ± 1.33	$17.24 \pm 0.88 (73.8 \pm 0.6)$	$5.04 \pm 0.37 (20.2 \pm 0.5)$	$1.08 \pm 0.09 (5.7 \pm 0.2)$
0.1 Gy ^{56}Fe	21.13 ± 0.90	$15.61 \pm 0.65 (74.4 \pm 0.5)$	$4.34 \pm 0.22 (19.8 \pm 0.4)$	$1.17 \pm 0.07 (5.5 \pm 0.1)$
0.5 Gy ^{56}Fe	23.15 ± 1.05	$17.08 \pm 0.69 (74.4 \pm 0.6)$	$4.80 \pm 0.31 (19.7 \pm 0.5)$	$1.15 \pm 0.10 (5.4 \pm 0.1)$
2.0 Gy ^{56}Fe	22.66 ± 0.69	$16.61 \pm 1.13 (74.3 \pm 0.7)$	$4.80 \pm 0.44 (19.9 \pm 0.6)$	$0.96 \pm 0.13 (5.4 \pm 0.2)$
2.0 Gy ^{28}Si	22.33 ± 1.08	$16.66 \pm 0.75 (75.2 \pm 0.5)$	$4.49 \pm 0.29 (19.0 \pm 0.5)$	$1.19 \pm 0.07 (5.2 \pm 0.1)^e$

Values are mean $\pm$ SE; numbers in parentheses are the cell percentages. [a] Number of cells $\times 10^6$/ml; data obtained with the ABC Vet Hematology Analyzer. [b] $P < 0.001$ vs. 0 Gy; $P < 0.05$ vs. 0.5 Gy; [c] $P < 0.001$ vs. 0 Gy and; $P < 0.05$ vs. 0.1 Gy and 0.5 Gy; [d] $P < 0.05$ vs. 0 Gy; [e] $P < 0.05$ vs. 0 Gy and 0.1 Gy ^{56}Fe.

post hoc analysis showed fewer differences among groups than in blood. Mean values for CD4-to-CD8 cell ratios obtained for the 0, 0.1, 0.5, and 2.0 Gy groups, respectively, were as follows: 2.1 ± 0.1, 1.9 ± 0.03, 1.8 ± 0.1, and 2.4 ± 0.04 (blood) and 1.5 ± 0.1, 1.6 ± 0.1, 1.6 ± 0.1, and 1.9 ± 0.04 (spleen). The ratios were significantly dependent on dose in both blood and

spleen ($P < 0.001$); post hoc analysis showed that animals receiving 2.0 Gy had significantly higher values compared with all other groups in both body compartments. However, in blood, only the 0.5 Gy dose caused significantly higher ratios compared with 0 Gy and 0.1 Gy exposures. Significant proportional changes were also observed in both T cell subsets on *day 4* (Fig.
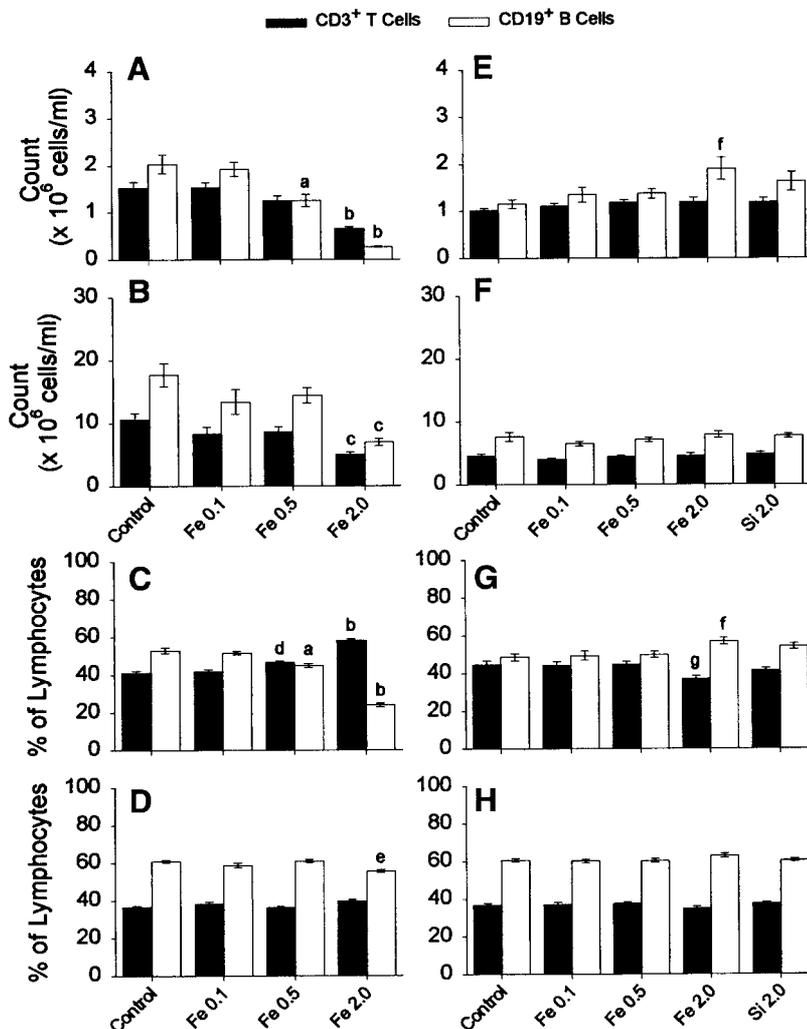

Fig. 3. T (CD3$^+$) and B (CD19$^+$) lymphocytes in blood (*A*, *C*, *E*, and *G*) and spleen (*B*, *D*, *F*, and *H*). *A–D*: *day 4*; *E–H*: *day 113*. Cells staining with fluorescent anti-CD3 or anti-CD19 antibodies within the gated lymphocyte population were quantified by flow cytometry analysis. Each bar represents the mean $\pm$ SE. [a] $P < 0.001$ vs. control and 0.1 Gy; [b] $P < 0.001$ vs. all other groups; [c] $P < 0.001$ vs. control and $P < 0.05$ vs. 0.1 Gy and 0.5 Gy; [d] $P < 0.005$ vs. control and 0.1 Gy; [e] $P < 0.005$ vs. control and 0.5 Gy; [f] $P < 0.05$ vs. control; [g] $P < 0.05$ vs. control and 0.5 Gy. Linear regression analysis: T cell percentage in blood = $41.457 + (8.505 \times \text{dose})$, $r^2 = 0.796$; B cell number in blood = $1.931 - (0.860 \times \text{dose})$, $r^2 = 0.604$; B cell percentage in blood = $52.763 - (14.459 \times \text{dose})$, $r^2 = 0.884$.
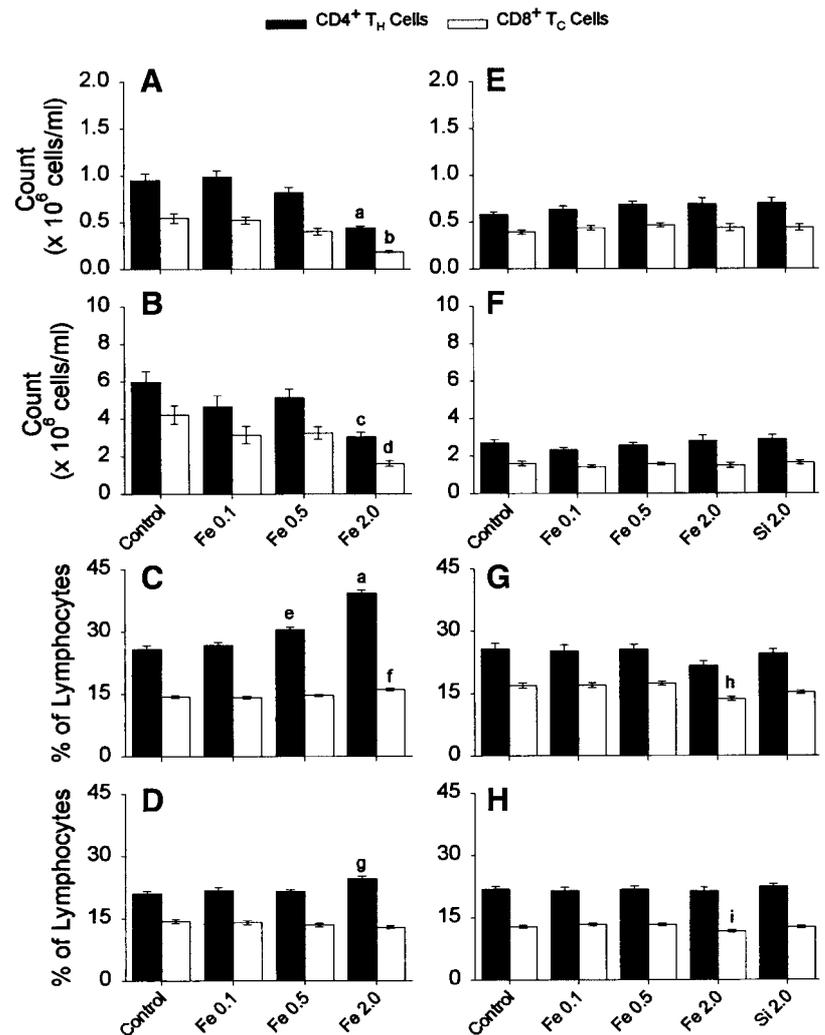
Fig. 4. T helper (T_H; $CD4^+$) cell and T cytotoxic (T_C; $CD8^+$) lymphocytes in blood (*A*, *C*, *E*, and *G*) and spleen (*B*, *D*, *F*, and *H*). *A–D*: *day 4*; *E–H*: *day 113*. Cells double-stained with fluorescent anti-CD3/anti-CD4 or anti-CD3/anti-CD8 antibodies within the gated lymphocyte population were quantified by flow cytometry analysis. Each bar represents the mean ± SE. [a]$P < 0.001$ vs. all other groups; [b]$P < 0.005$ vs. all other groups; [c]$P < 0.001$ vs. control and $P < 0.05$ vs. 0.5 Gy; [d]$P < 0.001$ vs. control and $P < 0.005$ vs. 0.1 Gy and 0.5 Gy; [e]$P < 0.01$ vs. control and 0.1 Gy; [f]$P < 0.001$ vs. control and 0.1 Gy and $P < 0.05$ vs. 0.5 Gy; [g]$P < 0.001$ vs. control and $P < 0.01$ vs. 0.1 Gy and 0.5 Gy; [h]$P < 0.001$ vs. control, 0.1 Gy and 0.5 Gy; [i]$P < 0.01$ vs. 0.1 Gy.

4). The dose-dependent increases in T_H cell percents were significant in blood and spleen ($P < 0.001$), although the increase was linear only in blood ($r^2 > 0.7$). Dose dependency for T_C cell percents was seen only in blood ($P < 0.001$).

By *day 113* (Fig. 4), a dose-dependent decrease in T_H cell percents was found in both blood ($P < 0.001$) and spleen ($P < 0.005$). In blood, the T_H cell proportion after 2.0 Gy ^{56}Fe was lower than after 0 Gy, 0.1 Gy, and 0.5 Gy; spleens from these mice had lower T_H cell percents than the 0.1 Gy group. A dose-dependent effect was noted in the CD4-to-CD8 cell ratio only in the spleen ($P < 0.01$), with the 2.0 Gy ^{56}Fe-irradiated mice having higher values than those exposed to 0.1 Gy. Means for the ratios in the 0, 0.1, 0.5, 2 Gy ^{56}Fe and 2 Gy ^{28}Si groups, respectively, were 1.5 ± 0.1, 1.5 ± 0.1, 1.5 ± 0.1, 1.6 ± 0.04, and 1.6 ± 0.1 (blood) and 1.7 ± 0.1, 1.6 ± 0.03, 1.6 ± 0.1, 1.8 ± 0.1, and 1.8 ± 0.04 (spleen).

Data in Fig. 5 show that on *day 4* there were no radiation dose-dependent changes in $NK1.1^+$ NK cell numbers in either body compartment. However, the percentages of these cells in both blood and spleen were dependent on dose ($P < 0.001$); linearity was evident

only in blood ($r^2 > 0.8$). By *day 113* (Fig. 5), NK cell numbers and percentages in the blood decreased in a dose-dependent manner ($P < 0.001$ for both measurements). Mice irradiated with 2.0 Gy ^{28}Si had significantly lower NK cell numbers compared with 0.1 ^{56}Fe and lower percents compared with the 0 Gy and 0.1 Gy ^{56}Fe groups.

Stem cell populations in the spleen. Flow cytometry analysis of splenocytes for stem cell populations was performed only on *day 113*. The results showed, as expected, very low numbers of these cells based on size and side scatter and presence of specific surface markers. Although there were no detectable cells expressing the CD34 marker without simultaneous expression of Ly-6, means ($\times 10^4$/ml) for $CD34^+$/$Ly6^+$ cells ranged from 0.31 ± 0.04 (2.0 Gy ^{28}Si) to 0.48 ± 0.07 (0 Gy). Means for $Ly-6^+$ cells ranged from 13.4 ± 0.9 (2 Gy ^{28}Si) to 14.9 ± 0.8 (0 Gy). There were no statistically significant differences among groups.

Spontaneous and mitogen-induced blastogenesis. The results showed that basal incorporation of [^{3}H]-TdR was radiation dose-dependent on *day 4* in both blood ($P < 0.001$) and spleen ($P < 0.05$) (Fig. 6). Paired comparisons of blood data showed that the 2.0 Gy
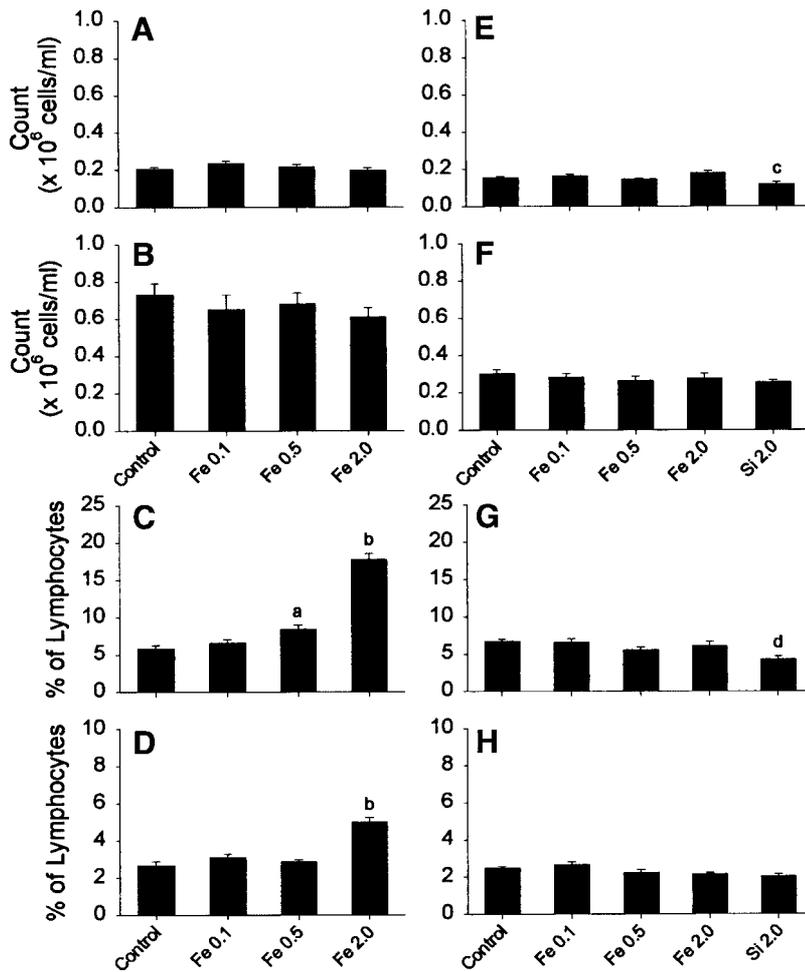
Fig. 5. Natural killer (NK1.1$^+$) cells in blood (*A, C, E,* and *G*) and spleen (*B, D, F,* and *H*). *A–D*: *day 4*; *E–H*: *day 113*. Cells stained with fluorescent anti-NK1.1 antibody within the gated lymphocyte population were quantified by flow cytometry analysis. Each bar represents the mean ± SE. [a]$P < 0.05$ vs. control; [b]$P < 0.001$ vs. control, 0.1 Gy, and 0.5 Gy; [c]$P < 0.05$ vs. 0.1 Gy; [d]$P < 0.005$ vs. control and 0.1 Gy. Linear regression analysis: blood %NK = 5.779 + (5.954 × dose), $r^2 =$ 0.830; spleen %NK = 2.684 + (1.114 × dose), $r^2 =$ 0.584.

group had significantly greater [^{3}H]TdR uptake compared with all other groups. In the spleen, this same group had higher values than the 0 Gy controls. On *day 113*, spontaneous blastogenesis in the spleen was radiation dose dependent ($P < 0.05$); further analysis revealed that 2.0 Gy ^{28}Si-irradiated mice had lower counts/min values than those irradiated with 0.5 Gy ^{56}Fe.

Splenocyte response to PHA stimulation (Table 3) was affected by dose on *day 4* ($P < 0.01$) and post hoc comparisons showed that the 2.0 Gy ^{28}Si group had lower counts/min values than the nonirradiated controls. By *day 113*, the response to PHA and LPS was significantly dose dependent ($P < 0.05$ and $P < 0.01$, respectively). Further analysis showed that the 2.0 Gy
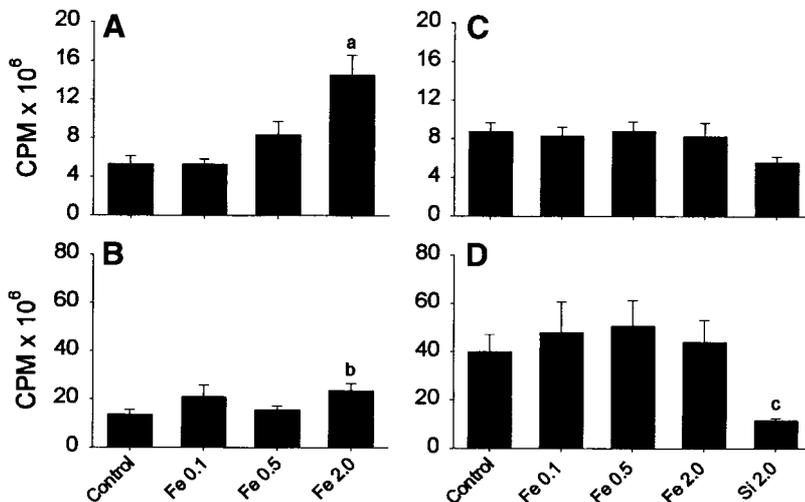

Fig. 6. Spontaneous blastogenesis of leukocytes in blood (*A* and *C*) and spleen (*B* and *D*). *A* and *B*: *day 4*; *C* and *D*: *day 113*. Each bar represents the mean ± SE. [a]$P < 0.001$ vs. control and 0.1 Gy and $P < 0.01$ vs. 0.5 Gy; [b]$P < 0.05$ vs. control; [c]$P < 0.05$ vs. 0.5 Gy.

Table 3. *Splenocyte response to mitogens and cytokine production*

Group	DNA synthesis, counts/min $\times 10^{4}$[a]			Cytokines, pg/ml[b]	
	PHA	ConA	LPS	IL-2	TNF-α
Day 4					
0 Gy	28.09 ± 3.98	37.59 ± 4.53	28.98 ± 1.82	77.1 ± 10.6	427.2 ± 49.2
0.1 Gy ^{56}Fe	15.72 ± 3.91	27.08 ± 4.79	30.26 ± 1.73	75.9 ± 7.7	365.2 ± 47.1
0.5 Gy ^{56}Fe	26.05 ± 3.23	39.74 ± 4.79	31.93 ± 1.91	68.9 ± 5.8	413.8 ± 43.2
2.0 Gy ^{56}Fe	13.61 ± 2.31[c]	26.47 ± 2.65	29.67 ± 1.27	67.5 ± 4.6	465.7 ± 62.2
Day 113					
0 Gy	21.99 ± 1.50	25.20 ± 2.31	30.77 ± 1.04	116.8 ± 12.2	228.7 ± 20.3
0.1 Gy ^{56}Fe	20.39 ± 1.62	20.36 ± 2.33	30.03 ± 1.20	117.2 ± 8.1	230.1 ± 21.1
0.5 Gy ^{56}Fe	20.47 ± 2.06	21.79 ± 3.08	28.92 ± 1.14	115.3 ± 13.8	247.4 ± 21.6
2.0 Gy ^{56}Fe	22.06 ± 2.46	24.87 ± 2.80	29.36 ± 0.80	115.6 ± 9.2	226.0 ± 12.6
2.0 Gy ^{28}Si	29.00 ± 2.83[d]	26.82 ± 3.07	34.28 ± 1.24[e]	97.5 ± 5.2	257.5 ± 19.4

Values are mean $\pm$ SE. [a] DNA synthesis in response to mitogens was quantified after 48-h incubation with mitogen and a pulse label with [^{3}H]thymidine ([^{3}H]TdR) during the last 4 h. Mean counts/min values for control wells containing no mitogen on *day 4* ranged from 0.16 ± 0.03 (0.1 Gy ^{56}Fe) to 0.25 ± 0.05 (0 Gy) and on *day 113* from 0.23 ± 0.02 (0 Gy) to 0.51 ± 0.19 (2.0 Gy ^{28}Si). [b] Cytokines were quantified in cell supernatants after a 48-h incubation with phytohemagglutinin (PHA) and no [^{3}H]TdR; assay sensitivities were 3 pg/ml (interleukin-2; IL-2) and 5.1 pg/ml (tumor necrosis factor-α; TNF-α). ConA, concanavalin A; LPS, lipopolysaccharide. [c] $P < 0.05$ vs. 0 Gy control at 4 days; [d] $P < 0.05$ vs. 0.5 Gy ^{56}Fe; [e] $P < 0.05$ vs. 2.0 Gy ^{56}Fe and $P < 0.01$ vs. 0.5 Gy ^{56}Fe.

^{28}Si group had higher counts/min values than the 0.5 Gy ^{56}Fe group for PHA and higher values for LPS compared with the 0.5 Gy ^{56}Fe and 2.0 Gy ^{56}Fe groups. There were no significant effects on SI values based on ANOVA or Tukey analysis (data not shown).

Cytokine production. The concentrations of IL-2 and TNF-α in supernatants of splenocytes stimulated with PHA are shown in Table 3. There was no radiation dose dependence on *day 4* for either cytokine and post hoc analysis revealed no significant differences among groups. On *day 113*, although the lowest level of IL-2 was produced by spleen cells from the 2.0 Gy ^{28}Si-irradiated group, no statistical significance was obtained for any of the comparisons for either cytokine.

Analysis of nonlymphoid cells in blood. The data on *day 4* showed a highly significant radiation dose effect ($P < 0.001$) for RBC counts, hemoglobin, and hematocrit (Fig. 7); a less pronounced dose effect was noted in MPV ($P < 0.05$). Pairwise comparisons indicated that the group receiving 2.0 Gy had lower RBC numbers than all other groups and that hemoglobin, hematocrit, and MPV were depressed compared with means obtained for the 0 Gy group. No significant differences were found for thrombocyte numbers or any of the other measurements. The ranges of means for data obtained on *day 4* not shown are presented here: platelets ($\times 10^{6}$/ml), 792 ± 21 (0 Gy) to 837 ± 34 (0.5 Gy); MPV (fl), 7.4 ± 0.1 (2.0 Gy) to 8.3 ± 0.3 (0 Gy); MCV (fl), 48.3 ± 0.3 (2.0 Gy) to 49.1 ± 0.3 (0.1 Gy); MCH (pg), 15.6 ± 0.1 (0 Gy) to 15.7 ± 0.1 (0.5 Gy); and MCHC (g/dl), 31.8 ± 0.2 (0.1 Gy) to 32.3 ± 0.1 (2.0 Gy). On *day 113*, a dose-dependent effect was seen in thrombocyte numbers ($P < 0.005$), and Tukey analysis showed that platelets were reduced after 2.0 Gy ^{28}Si irradiation compared with 0 Gy and 0.01 Gy ^{56}Fe. The ranges of means on *day 113* for data not shown are given here: platelets ($\times 10^{6}$/ml), 744 ± 30 (2.0 Gy ^{28}Si) to 931 ± 54 (0.1 Gy); MPV (fl), 9.4 ± 0.2 (0 Gy) to 9.8 ± 0.3 (2.0 Gy ^{28}Si); MCV (fl), 46.1 ± 0.2 (0.1 Gy) to 46.6 ± 0. (2.0 Gy ^{28}Si); MCH (pg), 15.2 ± 0.1 (0.2 Gy) to 15.4 ± 0.1 (2.0

Gy ^{28}Si); and MCHC (g/dl), 33.0 ± 0.1 (2.0 Gy ^{56}Fe) to 33.2 ± 0.1 (0.5 Gy).

DISCUSSION

In the present study, the *day 4* postexposure time was selected as the early time point of measurement, because it was close to the nadir in the types of immune parameters analyzed in our previous studies with low-LET radiation. The 113-day time point was selected, because immune reconstitution should be completed by then and any differences noted among groups would reflect long-term modification in immune system status. The effect of stress due to behavioral assessments in the long-term animals was minimized by allowing a 2-wk or greater interval before euthanasia on *day 113*. In addition, because nonirradiated control mice were subjected to the same behavioral assessments as the irradiated animals, any differences between the control and test groups in immunological status can be attributed to the effects of radiation exposure. Ideally, however, stress-induced changes due to behavioral testing should be controlled for by inclusion of a group that had not been subjected to these measurements.

Four days postirradiation with ^{56}Fe. Results show that total body mass was similar among the irradiated and nonirradiated groups. However, both the thymus (0.5 Gy and 2.0 Gy) and spleen (2.0 Gy) were significantly atrophied postirradiation. There are a number of factors that play a role in radiation-induced decline in mass, including cell death, changes in lymphocyte trafficking, and dehydration. Because there were no significant differences in body mass, dehydration is not likely to be a factor here. The greater dependence of thymus mass on dose, compared with the spleen, was somewhat surprising. In our past studies with γ-rays and protons (both are low LET), the spleen was more linearly dependent on dose than the thymus (14, 38). This suggests that the response of different lymphoid organs may be somewhat dependent on radiation qual-
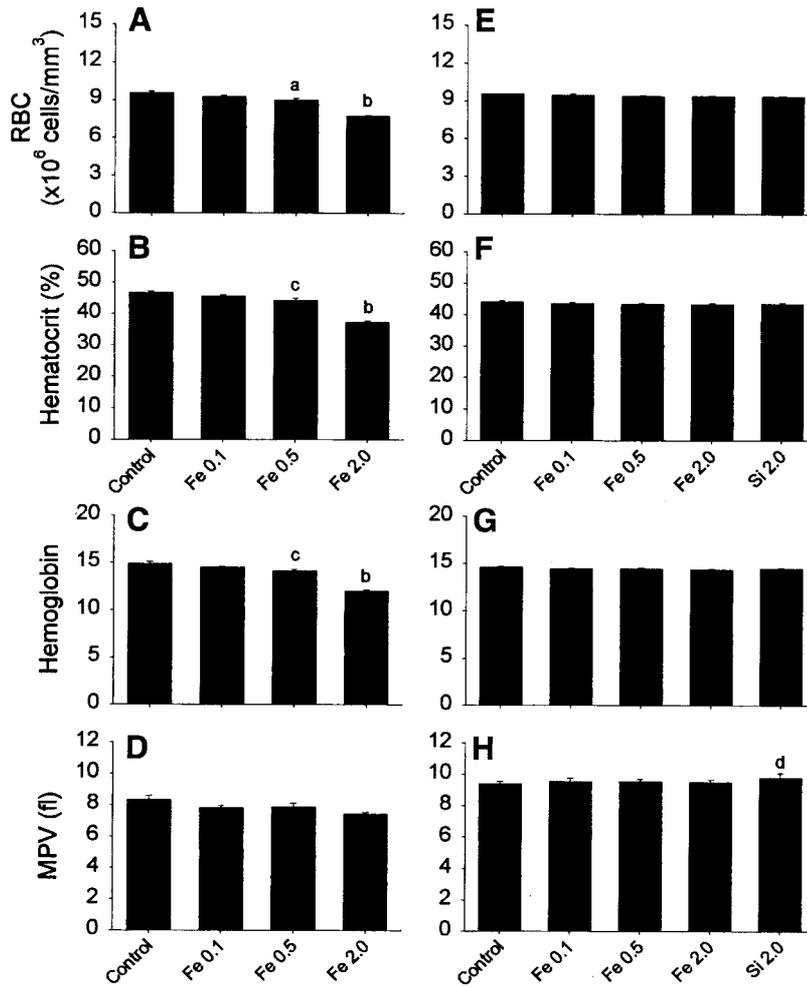
Fig. 7. Analysis of erythrocytes in blood. *A–D*: *day 4*; *E–H*: *day 113*. Each bar represents the mean ± SE obtained with the hematology analyzer. [a]$P < 0.005$ vs. control; [b]$P < 0.001$ vs. all other groups; [c]$P < 0.05$ vs. control; [d]$P < 0.05$ vs. control and $P < 0.005$ vs. 0.1 Gy. Linear regression analysis: red blood cell (RBC; *A* and *E*) = $9.455 - (0.866 \times \text{dose})$, $r^2 = 0.725$; hematocrit (*B* and *F*) = $46.381 - (4.519 \times \text{dose})$, $r^2 = 0.755$; hemoglobin (*C* and *G*) = $14.774 - (1.363 \times \text{dose})$, $r^2 = 0.739$. [See text for data on thrombocyte counts, mean platelet volume (MPV; *D* and *H*), mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration values.]

ity. Recent immunohistochemical studies of mouse mammary glands and skin have demonstrated that the extent of microenvironment remodeling is affected by the form of radiation and that the effects of γ-rays differ from those of ^{56}Fe (6, 10). Although there is little in the literature regarding the effects of iron ions on the thymus and spleen, studies (11, 16, 22) utilizing other forms of high-LET radiation, such as neutrons and fast carbon ions, have reported profound morphological and cellular changes after exposure. Additionally, although repair processes are clearly evident in lymphoid tissues within a week after exposure (22, 23), abnormal function can continue for a long time thereafter (42).

Splenic and blood leukocyte counts were highly dependent on ^{56}Fe radiation dose. Analysis showed that all three major leukocyte populations were significantly depleted in the blood from mice in the 2.0 Gy group. In the spleen, however, the 2.0 Gy dose reduced lymphocyte and granulocyte, but not monocyte-macrophage, numbers. The progressive increase in the percentages of monocyte/macrophages in blood and spleen with increasing radiation dose, despite no significant differences in absolute numbers among the groups, reflects the relatively greater radiosusceptibility (and thus greater depletion) of other leukocyte

populations. In addition, differences in radiosensitivity have been previously noted with the same cell type in different body locations. In the case of monocyte-macrophages exposed to X-rays, those residing in the bone marrow, lymph nodes, and peritoneal cavity are fairly radioresistant (30, 33), whereas those in lung alveoli are radiosensitive (40). Interestingly, the radiation-dependent decrease in blood monocyte percents found here differs from our previous observation that proton irradiation up to 3 Gy does not significantly affect the proportion of blood monocytes (14). In contrast to monocytes, 2 Gy caused a significant decrease in granulocytes in the both the blood and spleen. Studies with mice exposed to γ-rays show that neutropenia occurs at 5–6 days postirradiation (20). In our previous study with protons, only a slight trend for a decrease in this population was noted in the blood on *day 4* postexposure to 3 Gy. These results suggest that high-LET radiation may shift the nadir of peripheral neutropenia to an earlier time point. To our knowledge, this is the first time a radiation-induced change in splenic granulocyte counts and percents have been presented after whole body exposure to high-LET radiation.

Relative radiosensitivities of the various lymphocyte populations (B > T > NK) and T cell subsets (CD8 > CD4) reported here are consistent with our previous

observations as well as those of others using γ-rays (5, 17, 38, 41) and protons (14). This suggests that data obtained with low-LET radiation may be predictive of the effects of high-LET radiation for these types of measurements. However, compartmental differences were again noted. For example, although the CD4-to-CD8 cell ratio was significantly dose dependent in both the blood and spleen, it was significantly increased at lower doses in the blood (0.5 Gy) than in the spleen (2 Gy). This pattern is similar to what we found with protons, i.e., the slope of the linear dose dependence was greater in the blood than in the spleen (14). However, after exposure to γ-radiation at similar doses, the slope of the CD4/CD8 dose dependence was greater in the spleen than in the blood (38). Furthermore, in the present study, there were no dose-dependent changes in either body compartment for NK cell counts. In contrast, irradiation with protons caused a slight, but reliable, dose-dependent decrease in splenic NK cells (14, 27).

Increases in basal DNA synthesis after irradiation found here are generally consistent with our previous observations with γ-rays and protons. However, irradiation with 2.0 Gy ^{56}Fe significantly depressed PHA-induced proliferation; with γ-rays, although the response to PHA and ConA (both are T cell mitogens) was similar among all groups, a significant dose-dependent decline occurred in the response to LPS (B cell mitogen) (15). With proton radiation, significant depression was noted in the response to all three mitogens (36). In addition, the present data show no change in IL-1β and TNF-α production by PHA-stimulated splenocytes from irradiated animals in the time course studied, whereas the study with γ-rays showed a significant dose-dependent decrease in IL-2, but not TNF-α, secretion (15). The cross-linking of glycoproteins present on lymphocytes by mitogens sends a cascade of signals through the cytoplasm to the nucleus, thereby inducing DNA synthesis, cell division, and secretion of cytokines. Thus our findings suggest that radiation quality may have differential effects on certain functional properties of lymphocytes, such as signal transduction and/or the ability to synthesize surface molecules involved in mitogen binding.

The reduction in RBC counts, hemoglobin, and hematocrit observed in the present study is similar to what we have previously found with γ-rays and protons. However, our γ-irradiated mice also exhibited dose-dependent changes in erythrocyte volume and MCHC (15) and proton-irradiated animals also had dose-related changes in MCV (mean erythrocyte volume), RDW (RBC distribution width), and MCHC (36). Lack of an effect on thrombocyte numbers found here is similar to our findings in the γ-irradiated mice; interestingly, proton irradiation resulted in increasing platelet numbers with increasing dose.

It is possible that the discrepancies noted above at 4 days after ^{56}Fe-, proton-, and γ-irradiation may be reflective of differences in radiation quality. However, they may also be partly due to the limited time course and different dose ranges used in these studies. The proton and γ-ray studies examined doses ranging from 0.5 to 3 Gy, whereas the study presented here examined doses up to only 2 Gy, suggesting that there may be a threshold between 2 and 3 Gy for some of the measured parameters. There are no other studies utilizing ^{56}Fe irradiation, that we are aware of, with which these findings can be directly compared. The majority of reports comparing low- and high-LET radiation effects on bone marrow-derived cells have focused on cytogenetic evaluations of lymphocytes after in vitro exposure. Among the most prominent examples are the investigations of Durante, Yang, and colleagues, (8, 9, 51, 52) demonstrating that significant differences in certain aspects of DNA damage and repair do indeed exist among radiations of differing quality.

113 Days postirradiation with ^{56}Fe and ^{28}Si. The acute effects of ^{56}Fe seen at 4 days postirradiation were no longer evident at 113 days. However, in the blood from mice receiving 2 Gy, B lymphocyte numbers and proportions were significantly increased and the total T and CD8$^+$ T$_C$ cell proportions were low in both the blood and spleen compared with the nonirradiated control group evaluated at this same time point. These findings suggest that ^{56}Fe irradiation may have compromised cell-mediated or adaptive immune responses that are important in defense against viral infections and immune surveillance against neoplastic cells. On the other hand, optimal antibody production may not be compromised due to sufficient numbers of B cells and CD4$^+$ T helper (T$_H$) cells. However, it remains to be determined whether the functions of these latter two populations are adequate in defense and whether the balance of T$_H$1 versus T$_H$2 cytokines provided by the CD4$^+$ cells results in normal immunoregulation. It is also unclear whether the B cells present at this time point are more radioresistant, being progeny of surviving precursors, than their predecessors.

There is considerable interest in silicon beams, because they have a depth-dose profile considered to be optimal for maximizing high-LET particle effects (47). Somewhat surprisingly, the long-term effects seen with 2.0 Gy ^{56}Fe irradiation on B and CD8$^+$ T$_C$ cells were not observed with 2.0 Gy ^{28}Si exposure in the present study. However, ^{28}Si irradiation resulted in somewhat increased responsiveness to PHA and LPS and lower numbers and percentages of NK cells in both the blood and spleen. The NK cells are important in the control of virus proliferation early after infection and also secrete cytokines (e.g., interferon-γ, interleukin-12, and others) that upregulate T cell responses. The low basal DNA synthesis by splenocytes after 2.0 Gy ^{28}Si irradiation is consistent with our finding that the mice in this group also had the lowest levels of Ly-6$^+$ and CD34$^+$/Ly-6$^+$ stem cells. Thus it appears that exposure to ^{28}Si may result in chronic immune dysfunction.

Perspectives

Many studies show that high-LET radiations are more effective and less dependent on dose rate than

γ-rays in life-shortening and tumorigenic effects (47), and that variations exist in DNA damage and repair (8, 9, 51, 52). The present study, which utilizes whole body irradiation with heavy particles, contributes novel information to understanding the effects of radiation on the immune system. The data demonstrate that the acute effects of high-LET radiation on lymphoid organs and bone marrow-derived cells are not always consistent with what has been demonstrated previously for low-LET radiations such as protons and γ-rays. Therefore, results from low-LET radiation studies may not apply to high-LET radiation effects; attempts at extrapolation could lead to erroneous conclusions. In addition, differing long-term effects were noted between ^{56}Fe and ^{28}Si, both of which are high LET. Thus not only LET but also other intrinsic qualities of a particle beam may be important. Furthermore, most immunological studies to date have evaluated the effects of exposure from a single source of radiation (i.e., only protons, for example, not protons combined with heavy ions). The present findings suggest that simultaneous exposure to radiations of differing quality, as would occur during extended spaceflight, could affect a broader range of immunological parameters than what has been reported for exposure to radiation from a single source. This implies that immune dysfunction due to irradiation in space could be more profound than is currently estimated, due to an increased spectrum of effects. Efforts should be undertaken to simulate the space radiation environment to test this possibility. It should be emphasized that the data presented here are relatively limited by doses, dose rate, and time course and are derived from a single mammalian system. Many questions remain to be answered. For example, it is still to be determined whether the observed immunomodulation after irradiation results in significantly impaired immune defenses against infectious agents, which may be problematic in space and whether exposure to multiple forms of radiation results in additive or synergistic deleterious effects. Studies currently ongoing in our laboratories will begin to answer some of these questions.

REFERENCES

1. **Alpen EL, Powers-Risius P, Curtis SB, and DeGuzman R.** Tumorigenic potential of high-Z, high-LET charged-particle radiations. *Radiat Res* 136: 382–391, 1993.
2. **Barendsen GW.** The relationships between RBE and LET for different types of lethal damage in mammalian cells: biophysical and molecular mechanisms. *Radiat Res* 139: 257–270, 1994.
3. **Bikle DD, Harris J, Halloran BP, and Morey-Holton ER.** Altered skeletal pattern of gene expression in response to space-flight and hindlimb elevation. *Am J Physiol Endocrinol Metab* 267: E822–E827, 1994.
4. **Blakely EA and Fry RJ.** Radiation protection in space. *Radiat Environ Biophys* 34: 129–131, 1995.
5. **Chambers KA, Harrington NP, Ross WM, and Filion LG.** Relative alterations in blood mononuclear cell populations reflect radiation injury in mice. *Cytometry* 31: 45–52, 1998.
6. **Costes S, Streuli CH, and Barcellos-Hoff MH.** Quantitative image analysis of laminin immunoreactivity in skin basement membrane irradiated with 1 GeV/nucleon iron particles. *Radiat Res* 154: 389–397, 2000.
7. **Curtis S and Letaw J.** Galactic cosmic rays and cell-hit frequencies outside the magnetosphere. *Adv Space Res* 9: 292–298, 1989.
8. **Durante M, Furusawa Y, George K, Gialanella G, Greco O, Grossi G, Matsufuji N, Pugliese M, and Yang TC.** Rejoining and misrejoining of radiation-induced chromatin breaks. IV. Charged particles. *Radiat Res* 149: 446–454, 1998.
9. **Durante M, George K, and Yang TC.** Biodosimetry of ionizing radiation by selective painting of prematurely condensed chromosomes in human lymphocytes. *Radiat Res, Suppl* 148: S45–S50, 1997.
10. **Ehrhart EJ, Gillette EL, and Barcellos-Hoff MH.** Immunohistochemical evidence of rapid extracellular matrix remodeling after iron-particle irradiation of mouse mammary gland. *Radiat Res* 145: 157–162, 1996.
11. **Erofeeva LM, Sapin MR, and Grigorenko DE.** The thymus of mice at different periods after irradiation with fast carbon ions. *Morfologiia* 117: 42–46, 2000.
12. **Fry RJM, JD, Boice J, Curtis SB, Bond VP, Grahn D, and Todd PW.** *Guidance on radiation received in space activities*. Bethesda, MD: National Council on Radiation Protection and Measurements, 1989, p. 152–163.
13. **Gridley DS, Andres ML, and Slater JM.** Enhancement of prostate cancer xenograft growth with whole-body radiation and vascular endothelial growth factor. *Anticancer Res* 17: 923–928, 1997.
14. **Gridley DS, Pecaut MJ, Dutta-Roy R, and Nelson GA.** Dose and dose rate effects of whole-body proton irradiation on leukocyte populations and lymphoid organs. Part I. *Immunol Lett* 80: 55–66, 2002.
15. **Gridley DS, Pecaut MJ, Miller GM, Moyers MF, and Nelson GA.** Dose and dose-rate effects of whole-body γ-irradiation: II. Hematological variables and cytokines. *In Vivo* 15: 209–216, 2001.
16. **Grigorenko DE, Sapin MR, and Erofeeva LM.** Splenic lymphoid tissue of mice after irradiation with fast carbon ions. *Morfologiia* 114: 80–84, 1998.
17. **Harrington NP, Chambers KA, Ross WM, and Filion LG.** Radiation damage and immune suppression in splenic mononuclear cell populations. *Clin Exp Immunol* 107: 417–424, 1997.
18. **Hau DM, Feng Y, Chen WC, Lin IH, Chen KT, Lin SS, and Wang MI.** Effects of gypenosides on cellular immunity of γ-ray-irradiated mice. *Chin Med J (Engl)* 106: 143–146, 1996.
19. **Heilmann J, Rink H, Taucher-Scholz G, and Kraft G.** DNA strand break induction and rejoining and cellular recovery in mammalian cells after heavy-ion irradiation. *Radiat Res* 135: 46–55, 1993.
20. **Hellmann S.** Principles of cancer management: radiation therapy. In: *Cancer: Principles and practice of oncology* (5th ed.), edited by DeVita VT, Hellman S, and Rosenberg SA. Philadelphia, PA: Lippincott-Raven, 1997, p. 307–332.
21. **Hsu HY, Yang JJ, and Lin CC.** Effects of oleanolic acid and ursolic acid on inhibiting tumor growth and enhancing the recovery of hematopoietic system postirradiation in mice. *Cancer Lett* 111: 7–13, 1997.
22. **Huiskamp R and VanEwijk W.** Repopulation of the mouse thymus after sublethal fission neutron irradiation. I. Sequential appearance of thymocyte subpopulations. *J Immunol* 134: 2161–2169, 1985.
23. **Huiskamp R, VanVliet E, and VanEwijk W.** Repopulation of the mouse thymus after sublethal fission neutron irradiation. II. Sequential changes in the thymic environment. *J Immunol* 134: 2170–2178, 1985.

24. **Ishihara H, Tsuneoka K, Dimchev AB, and Shikita M.** Induction of the expression of the interleukin-1β gene in mouse spleen by ionizing radiation. *Radiat Res* 133: 321–326, 1993.

25. **Jahns G, Meylor J, Fast T, Hawes N, and Zarow G.** Rodent growth, behavior, and physiology resulting from flight on the Space Life Sciences-1 Mission. 43rd Congress of the International Astronautical Federation. Washington, DC: 1992, p. 1–8.

26. **James SJ, Enger SM, and Makinodan T.** DNA strand breaks and DNA repair response in lymphocytes after chronic in vivo exposure to very low doses of ionizing radiation in mice. *Mutat Res* 249: 255–263, 1991.

27. **Kajioka EH, Andres ML, Li J, Mao XW, Moyers MF, Nelson GA, Slater JM, and Gridley DS.** Acute effects of whole-body proton irradiation on the immune system of the mouse. *Radiat Res* 153: 587–594, 2000.

28. **Kajioka EH, Andres ML, Mao XW, Moyers MF, Nelson GA, and Gridley DS.** Hematological and TGF-β variations after whole-body proton irradiation. *In Vivo* 14: 703–708, 2001.

29. **Kajioka EH, Gheorghe C, Li J, Andres ML, Folz-Holbeck J, Abell GA, Slater JM, Nelson GA, and Gridley DS.** Effects of proton and gamma radiation on lymphocyte populations and acute response to antigen. *In Vivo* 13: 525–533, 1999.

30. **Kornfeld L and Greenman V.** Effects of total-body x-irradiation on peritoneal cells of mice. *Radiat Res* 29: 433–444, 1966.

31. **Magee J and Chatterjee A.** Radiation chemistry of heavy-particle tracks. I. General considerations. *J Phys Chem* 84: 3529–3536, 1980.

32. **Mei MT, Craise LM, and Yang TC.** Induction of proline prototrophs in CHO-K1 cells by heavy ions. *Int J Radiat Biol Relat Stud Phys Chem Med* 50: 213–224, 1986.

33. **Meyer OT and Dannenberg AM Jr.** Radiation, infection, and macrophage function. II. Effect of whole body radiation on the number of pulmonary alveolar macrophages and their levels of hydrolytic enzymes. *J Reticuloendothel Soc* 7: 79–90, 1970.

34. **Nogami M, Huang JT, James SJ, Lubinski JM, Nakamura LT, and Makinodan T.** Mice chronically exposed to low dose ionizing radiation possess splenocytes with elevated levels of HSP70 mRNA, HSC70 and HSP72 and with an increased capacity to proliferate. *Int J Radiat Biol* 63: 775–783, 1993.

35. **Parsons JL and Townsend LW.** Interplanetary crew dose rates for the August 1972 solar particle event. *Radiat Res* 153: 729–733, 2000.

36. **Pecaut MJ, Gridley DS, Smith AL, and Nelson GA.** Dose and dose rate effects of whole-body proton-irradiation on lymphocyte blastogenesis and hematological variables. Part II. *Immunol Lett* 80: 67–73, 2002.

38. **Pecaut MJ, Nelson GA, and Gridley DS.** Dose and dose-rate effects of whole-body γ-irradiation: I. Lymphocytes and lymphoid organs. *In Vivo* 15: 195–208, 2001.

39. **Pecaut MJ, Simske SJ, and Fleshner M.** Spaceflight induces changes in splenocyte subpopulations: effectiveness of ground-based models. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R2072–R2078, 2000.

40. **Peel DM and Coggle JE.** The effect of X irradiation on alveolar macrophages in mice. *Radiat Res* 81: 10–19, 1980.

41. **Rowley DA, Kelley WA, and Manders JH.** Flow cytometric analysis of lymphocyte surface markers following a 1-Gy dose of gamma radiation. *Aviat Space Environ Med* 64: 528–533, 1993.

42. **Sapin MR, Erofeeva LM, and Grigorenko DE.** Response of immune system and lymphoid tissue of respiratory and gastrointestinal organs to space flight factors. *Vestn Ross Akad Med Nauk* 12: 41–45, 1999.

43. **Setlow R, Dicello JF, Fry RJM, Little JB, Preston RJ, Smathers JB, and Ullrich RL.** *Radiation Hazards to Crews of Interplanetary Missions: Biological Issues and Research Strategies*. Washington, DC: Space Studies Board, National Research Council & National Academy, 1996, p. 13–34.

44. **Shankar B, Premachandran S, Bharambe SD, Sundaresan P, and Sainis KB.** Modification of immune response by low dose ionizing radiation: role of apoptosis. *Immunol Lett* 68: 237–245, 1999.

45. **Simonsen LC, Cucinotta FA, Atwell W, and Nealy JE.** Temporal analysis of the October 1989 proton flare using computerized anatomical models. *Radiat Res* 133: 1–11, 1993.

46. **Sonnenfeld G, Mandel AD, Konstantinova IV, Berry WD, Taylor GR, Lesnyak AT, Fuchs BB, and Rakhmilevich AL.** Spaceflight alters immune cell function and distribution. *J Appl Physiol* 73: 191S–195S, 1992.

47. **Tobias CA, Blakely EA, Alpen EL, Castro JR, Ainsworth EJ, Curtis SB, Ngo FQ, Rodriguez A, Roots RJ, Tenforde T, and Yang TC.** Molecular and cellular radiobiology of heavy ions. *Int J Radiat Oncol Biol Phys* 8: 2109–2120, 1982.

48. **Todd P.** Heavy-ion irradiation of cultured human cells. *Radiat Res, Suppl* 7: 196–207, 1967.

49. **Todd P, Pecaut MJ, and Fleshner M.** Combined effects of space flight factors and radiation on humans. *Mutat Res* 430: 211–219, 1999.

50. **Williams JR, Zhang Y, Zhou H, Osman M, Cha D, Kavet R, Cuccinotta F, Dicello JF, and Dillehay LE.** Predicting cancer rates in astronauts from animal carcinogenesis studies and cellular markers. *Mutat Res* 430: 255–269, 1999.

51. **Wu H, Durante M, George K, and Yang TC.** Induction of chromosome aberrations in human cells by charged particles. *Radiat Res, Suppl* 148: S102–S107, 1997.

52. **Wu H, George K, and Yang TC.** Estimate of the frequency of true incomplete exchanges in human lymphocytes exposed to 1 GeV/u Fe ions in vitro. *Int J Radiat Biol* 75: 593–599, 1999.

53. **Yang TC, Craise LM, Mei MT, and Tobias CA.** Neoplastic cell transformation by heavy charged particles. *Radiat Res, Suppl* 8: S177–S187, 1985.

54. **Yang TC, Georgy KA, Craise LM, and Durante M.** Initiation of oncogenic transformation in human mammary epithelial cells by charged particles. *Radiat Oncol Investig* 5: 134–138, 1997.

55. **Zeitlin C, Heilbronn L, and Miller J.** Detailed characterization of the 1087 MeV/nucleon iron-56 beam used for radiobiology at the alternating gradient synchrotron. *Radiat Res* 149: 560–569, 1998.

56. **Zeitlin C, Heilbronn L, Miller J, Rademacher SE, Borak T, Carter TR, Frankel KA, Schimmerling W, and Stronach CE.** Heavy fragment production cross sections from 1.05 GeV/nucleon ^{56}Fe in C, Al, Cu, Pb, and CH2 targets. *Phys Rev C Nucl Phys* 56: 388–397, 1997.