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Effects of High- and Low-LET Radiation on Human Hematopoietic System Reconstituted in Immunodeficient Mice

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Abstract

Over the last 50 years, a number of important physiological changes in humans who have traveled on spaceflights have been catalogued. Of major concern are the short- and long-term radiation-induced injuries to the hematopoietic system that may be induced by high-energy galactic cosmic rays encountered on interplanetary space missions. To collect data on the effects of space radiation on the human hematopoietic system *in vivo*, we used a humanized mouse model. In this study, we irradiated humanized mice with 0.4 Gy of 350 MeV/n ²⁸Si ions, a dose that has been shown to induce tumors in tumor-prone mice and a reference dose that has a relative biological effectiveness of 1 (1 Gy of 250-kVp X rays). Cell counts, cell subset frequency and cytogenetic data were collected from bone marrow spleen and blood of irradiated and control mice at short-term (7, 30 and 60 days) and long-term (6–7 months) time points postirradiation. The data show a significant short-term effect on the human hematopoietic stem cell counts imparted by both high- and low-LET radiation exposure. The radiation effects on bone marrow, spleen and blood human cell counts and human cell subset frequency were complex but did not alter the functions of the hematopoietic system. The long-term data acquired from high-LET irradiated mice showed complete recovery of the human hematopoietic system in all hematopoietic compartments. The combined results demonstrate that, in spite of early perturbation, the longer term effects of high-LET radiation are not detrimental to human hematopoiesis in our system of study.

INTRODUCTION

The main goal of the NASA radiation program is to reduce the uncertainties in space radiation risk projections for cancer and tissue degeneration. Over the last 50 years, a number of important physiological changes to humans who have been onboard spaceflights have been catalogued (1–3) (see also NCRP reports no. 132 and 153). Of major concern are the short- and long-term radiation-induced injuries to the hematopoietic system, since the hematopoietic compartment is one of the most radiosensitive in the human body as a result

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of the presence of a large number of continuously and rapidly proliferating cells. The effect of exposure to the space environment is illustrated in studies that show changes not only in the immune response of T lymphocytes after spaceflight but also depletion of the numbers of T and B cells of crewmembers of STS-41B and STS-41D (4). Altered differentiation of human bone marrow hematopoietic progenitor cells during the STS-63 and STS-69 missions were also observed (5). Studies in mice that were aboard STS-108 show hematologic changes of CD34⁺ cells, early blast cells and macrophage progenitors in the bone marrow (6). Mouse studies during the STS-118 mission revealed alterations in leukocyte subpopulations of the bone marrow and spleen (7–9). Other work in both nonhuman primates and mice shows a suppression of hematopoietic differentiation of macrophages and other blood cells (10–14). These in-flight studies are bolstered by ground-based findings, which suggest that high-linear energy transfer (LET) radiation, a component of galactic cosmic rays, poses a health threat to astronauts. In particular, missions beyond low-Earth orbit may be particularly detrimental to the immune system (15, 16). In addition, there are supporting “real world” forensic dosimetry findings of chromosomal damage in astronauts’ peripheral blood lymphocytes after long-term missions (17–20). Together, these data support a consensus that space radiation appears to impart important short- and long-term effects on the hematopoietic system (21, 22).

In this study, we collected data on the effects of high-LET radiation on different stages of human hematopoiesis *in vivo*. We used a humanized mouse model, whereby the effects of radiation on human hematopoiesis can be studied in different hematopoietic compartments. The focus of this study was on the collection of data that would allow us to build a detailed picture of the role of high-LET radiation on cell killing at different developmental stages starting with the hematopoietic stem cells (HSC). This data was complemented with developmental and frequency analysis of myeloid and lymphoid cell lineages in bone marrow, spleen and blood using multicolor flow cytometric immunophenotyping (FACS analysis). Furthermore, the study power was expanded to include the detection of aberrant cell populations that may suggest hematopoietic oncogenesis. Cytogenetic evaluation of the radiation effects was another aspect of the radiation effects that we also followed. Also, to achieve a better understanding of the ²⁸Si ion effects for these end points, we used X-ray radiation as reference.

MATERIALS AND METHODS

Humanized Mouse Generation

All animal husbandry and experimental procedures were conducted in accordance with applicable federal and state guidelines and approved by the Animal Care and Use Committees of Columbia University.

Immunodeficient NOD.Cg-*Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ* (NSG) mice (Jackson Laboratory, Bar Harbor, ME), aged 6 to 8 weeks, were engrafted with commercially available human cord blood CD34⁺ cells (Cincinnati Children’s Hospital Medical Center; Cincinnati, OH). For the engraftment, the mice received a 2.0 Gy dose of γ -ray radiation, followed by injection of 200,000 human CD34⁺ cells into the tail vein within 24 h postirradiation.

Twelve weeks later, engraftment was quantified using monoclonal antibodies (mAbs) specific to murine and human hematopoietic elements. To elucidate human hematopoietic reconstitution, detailed characterization of the engrafted cell populations was undertaken (listed below). For analysis of the different human cell populations from mouse bone marrow and spleen, the mice were euthanized, and peripheral blood, spleen, femurs and tibiae were obtained. The femurs and tibiae were flushed with DPBS, 5% BSA for bone marrow cell isolation. Single cell suspensions were made from bone marrow and spleen by passing the tissues through a 40- μ M cell strainer (Thermo Fisher Scientific™ Inc., Rockford, IL).

Mouse Irradiations

Irradiations were performed at the NASA Space Radiation Laboratory (NSRL) located at the Brookhaven National Laboratory (BNL; Upton, NY) with 0.4 Gy of 350 MeV/n ^{28}Si ions, previously shown to induce tumors in mice (23). For irradiations, the mice were shipped to the NSRL by commercial carrier, acclimatized for 3 days in the BNL animal facility and then irradiated or sham irradiated at NSRL. All X-ray irradiations were done at Columbia University Medical Center (New York, NY). A dose of 1 Gy X rays (250 kVp) was used. Radiation with these characteristics is accepted as having a relative biological effectiveness (RBE) of 1. The mice were in good health before and after irradiation, they were active and their behavior was normal. We did not encounter any loss of mice as result of the transportation or irradiation.

Human and Mouse Cell Isolation

Mouse blood was obtained either from the tail artery for engraftment analysis (an average of 50 μ l) or by cardiac puncture after the mice were euthanized. For analysis of the different human cell populations from mouse bone marrow and spleen, the mice were sacrificed and then spleen, femurs and tibiae were collected. The spleens were homogenized by passing through a 40 μ M cell strainer (Thermo Fisher Scientific) and then resuspended at 1 ml DPBS/2% FBS/16 IU/ml heparin. The bone marrow tissue was obtained from mouse femurs and tibiae by flushing of the bones using a syringe containing DPBS/5% BSA followed by passing of the tissue through a 40- μ M cell strainer. The final volume of the bone marrow samples was adjusted to 1 ml.

Immunophenotyping

Flow cytometry-based cell counting—All cell counts shown in this study, with the exception of the HSC counts, were obtained using flow cytometers with volumetric-based fluidics [BD Accuri™ (4-color machine; BD Biosciences, San Jose, CA) or Beckman Coulter® CytoFLEX (13-color machine) available at the laboratory of the PI and beads with standardized counts (SPHERO™ AccuCount Ultra Rainbow fluorescent particles, 5.0–5.9 μ m; Spherotech Inc., Lake Forest, IL). HSC counts were estimated with data from the flow sorters used to isolate these cells.

The following antibody panels were used for identification of different cell populations (shown as surface markers).

Aberrant human HSC/early progenitors—Anti-human CD45⁺, lin[−] (or negative for CD2, CD3, CD14, CD16, CD19, CD56 and CD235a), CD34⁺ and CD38[−].

Peripheral blood and spleen human cell analysis—CD45⁺, CD3⁺, CD20⁺, CD11b⁺.

Mouse cell analysis—anti-mouse CD45⁺, CD20⁺ and CD3⁺.

Multicolor flow cytometric immunophenotypic analysis of bone marrow and spleen samples from humanized mice to screen for developmental aberrancies and/or hematopoietic malignancies—Four specific 8-color panels consisting of anti-human antibodies were designed to study bone marrow and spleen samples. Analysis of normal mouse samples, to identify any potential cross-reaction between mouse cells and human antibodies confirmed no cross-reaction of the anti-human CD45 with mouse cells (results not shown). Preferential gating with human CD45 allowed clear separation and only CD45⁺ cells were investigated further. Detailed descriptions of the strategy used, representative printouts of bone marrow and spleen flow cytometric immunophenotyping data analysis and antibody data are shown in the Supplementary Figs. S5 and S6 and Table S2 (<http://dx.doi.org/10.1667/RR15148.1.S1>).

Spleen staining panels—Panel 1: CD5, CD10, CD19, CD20, CD34, CD45, kappa, lambda. Panel 2: CD2, CD3, CD4, CD5, CD7, CD8, CD25, CD45. This combination allows evaluation of maturing myeloid elements, monocytes, blasts, immature B cells, naive B cells and mature B cells (including shifts in kappa/lambda ratios), T cells (including shifts in CD4/CD8 ratios), NK cells as well as T-cell activation markers. In addition, the combination of these markers allows detection of the following aberrant populations: expanded and or aberrant myeloblast populations, atypical myeloid maturation, B-cell progenitor/B lymphoblasts (acute B-cell lymphoblastic leukemia/ lymphoma (B-ALL), T-cell progenitor/T lymphoblasts (acute T-cell lymphoblastic leukemia/lymphoma (T-ALL), numerous commonly encountered mature B-cell non-Hodgkin lymphomas (including but not limited to chronic lymphocytic leukemia/small lymphocytic lymphoma, mantle cell lymphoma, follicular lymphoma, marginal zone lymphoma and its variants, B-cell non-Hodgkin lymphoma, not otherwise specified, etc.), as well as a subset of mature T-cell lymphomas (including but not limited to peripheral T-cell lymphoma, NOS, adult T-cell lymphoma/leukemia, angioimmunoblastic T-cell lymphoma, etc.).

Bone marrow staining panels—Panel 1: CD10, CD33, CD34, CD38, CD45, CD117, CD123, HLA-DR. Panel 2: CD4, CD7, CD11b, CD13, CD15, CD34, CD45, CD64. This combination includes numerous myeloid and monocytoid markers as well as immaturity markers that allow the detailed evaluation of myeloid/granulocytic and monocytic maturation as well as aberrancies/discrepancies during maturation. In addition, mast cells, plasmacytoid dendritic cells and activated T cells can be characterized. Furthermore, these panels facilitate the detection of acute and chronic leukemia and myeloproliferative neoplasms (such as acute myeloid leukemia, acute monocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia etc.) and myelodysplastic syndromes.

The following flow cytometers were used for the analysis:

- The BD Accuri (4-color machine) and Beckman Coulter CytoFLEX (13-color machine) were used for peripheral blood engraftment analysis and for analysis of the short term X-ray effects on the HSC.
- The BD Canto II 8-color instrument was used for analysis of bone marrow and spleen samples from the humanized mice for developmental and tumor-related end points and for the analysis of the human HSC and progenitor cell development.
- The BD Aria™ II cell sorter and BD Influx™ cell sorter were used to sort bone marrow cells, HSC and lin[−] progenitors. FCS Express (De Novo™ Software, Los Angeles, CA) and FlowJo (Ashland, OR) were used for data analysis.

Cytogenetic Analysis

Chromosome dicentric analysis—Chromosomal aberrations in human lymphocytes were analyzed on days 7 and 60 postirradiation. For this, lymphocytes were isolated from the peripheral blood using Histopaque®–1083 (Sigma-Aldrich®, St. Louis, MO) and from spleen of humanized mice using red blood cell lysis solution (Thermo Fisher Scientific) and cultured in RPMI 1640 medium, supplemented with 15% fetal calf serum, antibiotics (penicillin/streptomycin) and 4% of phytohemagglutinin (PHA) (Gibco®, Grand Island, NY). For all cultures, 0.1 mg · ml^{−1} Colcemid™ (Gibco) was added 24 h after the culture was initiated for first metaphases arrest. The cultures were harvested at 52 h after setup using the standard treatment with hypotonic and Carnoy's fixative. Slides were stained with Leishman stain. Only human metaphases containing 46 centromeres were analyzed. Multicentric chromosomes (di-, tri-, tetra-,...) were recorded only when the corresponding number of acentric fragments were present. Polycentrics were converted into the equivalent number of dicentrics as (n − 1), where n is a number of centromeres. Chromosome spreads were manually analyzed with a Zeiss Axioplan2 microscope (Carl Zeiss, Jena, Germany) coupled to a Metafer® Slide Scanning System (MetaSystems, Altlussheim, Germany).

Chromosome mFISH—For mFISH analysis, bone marrow cells were isolated from mouse femurs. Human lineage negative CD45⁺, CD38[−], CD38⁺, CD34⁺ cells were isolated from the bone marrow using FACS Aria™ or Influx cell sorters (BD Biosciences). To obtain enough cell for the cytogenetic studies, 50,000–150,000 sorted cells were expanded in StemSpan™ SFEM II media, supplemented with human cytokines (Flt3L, SCF, IL-3 and IL-6) (STEMCELL™ Technologies Inc., Vancouver, Canada). Five days after culture setting up, 0.1 µg/ml Colcemid was added and the cells were harvested 4 h later using standard treatment with hypotonic and Carnoy's fixative. Two days later these slides were treated with 0.02 g/ml pepsin for 10 min before adding hybridization solution containing 24XCyte human probe-kit for (MetaSystems). This kit contains chromosome probes labeled with specific fluorochrome combinations for each chromosome. The slides were denatured at 73°C for 3 min and hybridized for 24 h at 37°C in ThermoBrite® System (Leica Biosystems, Nussloch, Germany). After washing and staining with DAPI, the slides were examined using fluorescent microscope (Carl Zeiss) with filter sets for FITC, Cy3.5, Texas Red, Cy5, Aqua and DAPI. Images were analyzed using ISIS mFISH imaging software (MetaSystems).

Bone Marrow Morphology—Humanized mouse femurs were obtained and fixed for 2 days in Cal-Ex II™ (Thermo Fisher Scientific). This solution allows simultaneous fixation and decalcification of bones. After fixation, the samples were paraffin embedded using standard methodologies. Serial sections (5-μm thickness) stained with hematoxylin and eosin (H&E) were sent for pathologic evaluation with clinical Zeiss Axio Lab.A1 laboratory microscope.

Statistical Analysis—To assess the potential statistical significance of radiation effects on various cell types in our mouse model system, we compared cell counts between irradiated and control mice, and in the same mice at different time points. To bring the error distribution closer to normal, we log-transformed these data. Statistical comparisons between means were performed using Student's *t* tests. Unpaired tests were applied to data on independent samples (e.g., irradiated vs. control mice), and paired ones were applied to situations where repeated measures were obtained (e.g., cell counts at different time points on the same mice). The commonly used type I error threshold of 0.05, with Bonferroni correction for multiple testing, was employed to indicate statistical significance. We also performed correlation analyses using the Pearson correlation coefficient and associated *P* values and confidence intervals.

RESULTS

Irradiations

To compare the effects of high-LET radiation to low-LET radiation, we used two radiation types at two different doses. For high-LET radiation we used 0.4 Gy of 350 MeV/n ²⁸Si ion, selected because of its previously demonstrated carcinogenic effect in tumor prone mice (23). This ion and energy delivered approximately 1.2 particles per cell (with estimated surface of 28 μm², or 6 μm diameter). As a radiation exposure benchmark, we used a 1 Gy dose of X rays (250 kVp), which exhibits an RBE of 1 at this dose and energy.

Assessment of Early and Late Effects of High-LET ²⁸Si Ions and X Rays on the Depletion and Recovery Kinetics of Human HSC in Humanized Mice

The array of cell types and relative population proportions of the human hematopoietic system are a function of the activity of HSC. Any effects on this multipotent cell population will have a large impact and downstream effects on immune function. Because cell killing and cell transformation are the most detrimental effects of radiation, we sought to establish the effects of high- and low-LET radiation on the survival of the human HSC *in vivo*.

For this, we examined radiation effects in three distinct cell populations in the mouse bone marrow (Fig. 1A): 1. total human CD45⁺ cells; 2. Lin[−] cells (CD45⁺/lin[−]; this population consists of cells that give rise to all blood lineages but does not express mature cell lineage markers; and 3. Lin[−] subpopulation defined by the CD45⁺/lin[−]/CD34⁺/CD38[−] markers. The latter subset includes the HSC, and multipotent progenitors (MPP); for simplicity, we will refer to this subset as HSC. We obtained the counts for these populations from bone marrow of 0.4 Gy 350 MeV/n ²⁸Si-ion-irradiated mice and control mice at days 7, 30 and 60 postirradiation. A total of 27 humanized mice were used for these experiments. The ratio of

the HSC counts of the control mice vs. irradiated mice were 10 ($P=0.007$) for day 7, 16 ($P=0.10$) for day 30 and 3 ($P=0.17$) at day 60. P values for days 30 and 60 are high despite the large ratios due to the presence of one or two outliers in the data. However, the comparison of the HSC fraction in the lin^- population between the irradiated and control cohorts show a significant effect of ^{28}Si ions (Fig. 1B). The ratio of the average frequencies of these cells in the control vs. irradiated mice was 5.4 for day 7 ($P=0.01$, $n_{\text{irr}}=6$, $n_{\text{ctr}}=4$), 4.4 for day 30 ($P=0.0009$, $n_{\text{irr}}=6$, $n_{\text{ctr}}=4$) and 4.5 for day 60 after irradiation ($P=0.035$, $n_{\text{irr}}=4$, $n_{\text{ctr}}=3$). An obvious next step was to assess the long-term effects of ^{28}Si ions. For this, we compared the HSC population in the bone marrow of 10 ^{28}Si irradiated mice and 12 control mice between the 6th and 7th months postirradiation. At this later time, no statistically significant difference between the irradiated and control mice was observed for these end points. (Fig. 1C).

To compare the short-term effects of the high-LET ^{28}Si ion to X ray radiation we used 11 humanized mice as X-ray radiation reference group. Seven of these mice received 1 Gy of X rays and the human HSC counts were compared between these mice and controls at days 7 and 60 postirradiation. The results show a very pronounced effect after X ray irradiation on the human HSC counts. No human HSC were detected by flow cytometry at day 7 postirradiation (Fig. 1D), even when the survey included $>3 \times 10^6$ bone marrow cells per flow cytometry sample. The HSC counts recovered at day 60 postirradiation but were lower than controls by a statistically significant 3.3-fold margin ($P=0.0042$).

No long-term experiments were performed using X-ray-irradiated mice. Overall, these results show significant depletion of human HSC *in vivo* in response to high-LET and low-LET exposures in the initial two months postirradiation. This is followed by HSC count recovery in the ^{28}Si irradiated mice 6–7 months postirradiation. Consequently, this raises an important question of how well the HSC can maintain the hematopoietic system at short intervals after irradiation.

Assessment of the Early and Late Effects of High-LET ^{28}Si Ions and X-Rays on the Human Cell Counts and Human Cell Development in Humanized Mice

To test the short- and long-term effects of 0.4 Gy 350 MeV/n ^{28}Si ions and 1 Gy X rays on the development of the human hematopoietic system in humanized mice, we performed extensive flow cytometric profiling of human cells from bone marrow and spleen from irradiated and control humanized mice. The goals of this analysis were twofold: 1. To find the effect of radiation on the early and late stages of differentiation of the human hematopoietic cells that can be seen in the bone marrow and spleen of humanized mice; and 2. To identify any aberrant groups of cells that may indicate a deviation of the normal developmental patterns or oncogenesis.

Short-term effects of ^{28}Si ions—Three groups consisting of 10 mice each (6 irradiated mice and 4 controls) were sacrificed at day 7, 30 or 60 after a dose of 0.4 Gy 350 MeV/n ^{28}Si ions and used for estimation of the bone marrow and spleen human cell counts, as well as for determining the differentiation patterns of the human cells in these organs.

A comparison of the human CD45⁺ hematopoietic cell counts in the bone marrow of ²⁸Si irradiated and control mice (unpaired *t* test, Fig. 2A) showed no statistically significant difference at day 7 (*P* = 0.33) or day 60 (*P* = 0.36) postirradiation although a downward trend in the CD45⁺ cells was observed in the irradiated cohort. At day 30, a statistically significant difference was observed for both human CD45⁺ cell counts (*P* = 0.02) and the human cell engraftment (*P* = 0.036). No differences were observed when the mouse CD45⁺ hematopoietic cell counts were compared between the irradiated and control groups.

A comparison of the human CD45⁺ hematopoietic cell counts in the spleen of ²⁸Si irradiated and control mice (unpaired *t* test, Fig. 2B) showed a statistically significant difference for all time points. In particular, the reduction of the human CD45⁺ cells at day 7 postirradiation, based on average cell counts, was threefold (*P* = 0.011), and 2.7-fold (*P* = 0.056) and 1.9-fold (*P* = 0.049) on days 30 and 60, respectively. These results indicate that spleen counts were affected by the ²⁸Si ions, in contrast to what we have observed in the bone marrow of these mice.

Short-term effects of X rays—For these experiments two groups consisting of 8 mice each (4 irradiated and 4 controls) were sacrificed at day 7 or 60 after 1 Gy X-ray irradiation (250 kVp). The comparison between the human CD45⁺ cell counts (irradiated vs. control mice) and mouse CD45⁺ cell counts (irradiated vs. control mice) in the bone marrow of X-ray irradiated and control mice (Fig. 2C), performed using unpaired *t* test analysis, showed no statistically significant cell count differences for either the day 7 or day 60 time points. The same comparison, performed for spleen cell counts (Fig. 2D), showed a statistically significant decrease of both human and mouse cell counts at day 7 postirradiation. No significant differences were observed at day 60 postirradiation for either cell type.

Developmental analysis of the human hematopoietic cells in the bone marrow of ²⁸Si-ion-irradiated mice—An example of this analysis with detailed explanation of the strategy used are shown in the Supplementary Fig. S5 (<http://dx.doi.org/10.1667/RR15148.1.S1>). Comparison of the frequencies of specific cell populations between irradiated and control mice (Supplementary Fig. S1), performed by unpaired *t* test analysis, showed the following statistically significant differences.

At day 7 postirradiation, there was a twofold decrease of the CD34⁺, CD117⁺ immature myeloid cells in the irradiated mice. At day 30 postirradiation, there was a 1.5-fold increase of the monocytes in the irradiated mice. At day 60 postirradiation, there was a 1.4-fold decrease of the lymphocytes and 1.5-fold increase of the monocytes in the irradiated mice. No differences were found for all other cell populations including the different stages of myeloid developments, B-cell development and the early progenitor populations, including the CD34⁺/CD38[−] and CD34⁺/CD38⁺ cells. No aberrant populations were found in any of the analyzed samples.

Subpopulation cell distribution in spleens of ²⁸Si irradiated mice—An example of this analysis with detailed explanation of the strategy used are shown in the Supplementary Fig. S6 (<http://dx.doi.org/10.1667/RR15148.1.S1>). The comparison of the frequencies of specific cell populations between irradiated and control mice (Supplementary

Fig. S2), performed using unpaired *t* test analysis, showed the following statistically significant differences.

Day 30: A sixfold decrease in the percentage of all B-cell populations including transitional B/maturing B/($P = 0.0007$) in the irradiated mice; a 1.2-fold decrease in CD5dim, CD20⁺ naive B cells ($P = 0.051$) in the irradiated mice; a 1.4-fold decrease in mature B cells ($P = 0.03$). Commensurate with this depressed B-cell population, the T-cell fraction/percentage (CD4⁺ and CD8⁺) increased twofold ($P = 0.003$).

Day 60: No differences in the CD20⁺, CD19⁺ maturing B ($P = 0.09$) but the CD5dim, CD20⁺ naive B cells were reduced 2.3-fold ($P = 0.01$) with mature B-cell percentages reduced twofold ($P = 0.009$) in the irradiated mice. T-cell percentages were twofold higher than the controls ($P = 0.007$).

Developmental analysis of the human hematopoietic cells in the bone marrow of X-ray-irradiated mice—Comparison of the frequencies of specific cell populations between irradiated and control mice (Supplementary Fig. S3; <http://dx.doi.org/10.1667/RR15148.1.S1>) performed using unpaired *t* test analysis showed the following statistically significant differences.

Day 7: Granulocytes up 1.7-fold, $P = 0.001$ in the irradiated mice. Day 60: Immature myelocytes down tenfold, $P = 0.02$, mature granulocytes down twofold down, lymphocytes up 1.4-fold up, $P = 0.02$, all in the irradiated mice. We did not identify any aberrant populations suggestive of oncogenic transformation in any of the samples that were analyzed.

Subpopulation cell distribution in spleens of X-ray-irradiated mice—Results from the spleen subpopulation analysis (Supplementary Fig. S4; <http://dx.doi.org/10.1667/RR15148.1.S1>) showed a significant effect of X rays on the B-cell populations. Day 7: A twofold decrease in the frequencies of CD5dim CD20⁺ naive B cells ($P = 0.005$) and a 2.4-fold increase in CD20⁺ CD19⁺ mature B cells ($P = 0.01$) in the irradiated mice. Conversely, the T-cell percentage (CD3⁺/CD7⁺) increased 2.4-fold ($P = 0.01$). On day 60, no statistically significant differences between irradiated and control mice were evident.

Long-term effects of 0.4 Gy 350 MeV/n ²⁸Si ions on the human cell counts and human hematopoietic cell frequencies in bone marrow and spleen of humanized mice—To assess the long-term effects of high-LET radiation on the human and mouse hematopoietic elements, CD45⁺ cells counts were obtained from a total of 63 humanized mice, (36 irradiated and 17 controls). These samples were collected during the 6th and 7th month postirradiation. The results (Fig. 3A and B) showed no statistically significant difference in the counts of the human hematopoietic cell populations between the irradiated and control groups, in both bone marrow and spleen. The analysis of the mouse hematopoietic cell counts shows a similar result. Similarly, likewise estimation of engraftment frequency of human cells into mouse bone marrow shows no statistically significant difference between the irradiated and the control group. And finally, there was no statistically significant difference in mouse weight between both groups (results not shown).

These findings point to the lack of long-term effects of high-LET radiation on the human and mouse cell counts in humanized mice.

Developmental analysis—The differentiation patterns of the engrafted human hematopoietic system were analyzed in a total of 63 mice, as stated above. Bone marrow and spleen cells were analyzed using the same antibody panels as describe above for the short-term analysis. The subpopulation analysis showed no statistically significant differences of the subpopulation frequencies between irradiated and control mice in both bone marrow and spleen (Figs. 4 and 5). Importantly, no malignancies or phenotypic aberrancies were detected. Long-term differentiation analysis was not performed in X-ray irradiated mice.

Assessment of the Early and Late Effects of High-LET ^{28}Si Ions and X rays on the Depletion and Recovery Kinetics of Human Peripheral Blood Cells in Humanized Mice

To obtain data on the short-term effects of high-LET radiation, we used 24 humanized mice exposed to 0.4 Gy 350 MeV/n ^{28}Si ions. Blood samples (~50 μl per mouse) were obtained 3 weeks prior to irradiation and at days 7, 30 and 60–75 postirradiation. We omitted human myeloid cells because their counts in humanized mouse peripheral blood are very low (approximately 5%). The depletion/recovery findings (Fig. 6A) showed the overall fluctuations of the blood cell counts for each mouse. We observed a statistically significant decrease (paired sample t test, $P = 0.004$) of the peripheral blood mononuclear cell (PBMC) counts (human CD45^+ cells) at day 7 postirradiation. This is attributable to the reduction in B-cell counts, which are the most radiation-sensitive human blood cells and comprise the majority of circulating lymphocytes in the humanized mouse. Remarkably, there was no statistically significant difference between the counts of PBMC and the B cells preirradiation and at day 30 or 60–75 postirradiation (Fig. 6B). We did find a statistically significant increase between preirradiation T-cell counts and those at day 30 or 75 postirradiation, which is normal for this model; this was observed in the control mice as well (Fig. 6C). These results indicate an early effect of high-LET radiation, but also fast recovery detected at days 30 and 60 postirradiation.

The short-term effect of X rays was studied in a cohort of 23 humanized mice that were 1 Gy X-ray irradiated (250 kVp). The human cell counts were assessed at days 7, 30 and 60 postirradiation. Surprisingly, the results showed a pronounced effect of X ray radiation on blood cell counts. The human PBMC numbers were reduced approximately fivefold at day 30 postirradiation compared to the preirradiation counts for each subject (Fig. 6D). This effect was primarily related to the B-cell losses. The number of B cells recovered slightly at day 30 postirradiation but decreased again at day 60 (Fig. 6E). The effect of X ray radiation was less pronounced on the T-cell population, where the average reduction of cell counts was only twofold (Fig. 6F).

To assess the long-term effects of high-LET radiation on the human and mouse blood cell counts, we obtained data from 53 humanized mice, (36 irradiated and 17 controls) between 6 and 8 months postirradiation. These results (Fig. 7A) showed no statistically significant difference in the human blood cell counts (human CD45^+ cells) between the irradiated and control groups ($P = 0.1$). The analysis of mouse PBMC (mouse CD45^+ cells) showed similar

results. Likewise, the estimation of engraftment frequency of human blood cells in the mouse blood showed no statistically significant difference between the irradiated and control groups. These results indicate that both human and mouse blood cell populations can recover after high-LET ^{28}Si -ion irradiation.

The long-term effects of X rays on human hematopoietic system were investigated in a separate cohort of X-ray-irradiated-humanized mice (16 irradiated and 12 control mice) where the blood cell counts were assessed 4 months after exposure to 1 Gy dose of X-ray radiation. The results (Fig. 7B) showed a 2.5-fold ($P = 0.007$) reduction in human PBMC compared to the control group, 3.3-fold ($P = 0.01$) reduction in human B-cell counts and 2.2-fold ($P = 0.05$) reduction in T-cell counts. The engraftment frequency of the human cells was at an average 24% for the irradiated cohort compared to 56% for the control group. No statistically significant difference was detected between the mouse CD45^+ cell counts (irradiated vs. control mice).

Together, these results indicate that with respect to blood cell killing, a dose 0.4 Gy 350 MeV/n ^{28}Si ion is less effective than a dose 1 Gy 250-kVp X ray. This was a surprising result, requiring additional data to be confirmed. Such data based on cytogenetic analysis of stem cells is presented below.

Cytogenetic Evaluation of the Chromosomal Damage Induced In Vivo by Doses of High- and Low-LET Radiation

For these experiments, we used two cytogenetic methodologies: dicentric chromosome formation and chromosome translocations.

Dicentric chromosome formation: exclusion of whole blood culture—To obtain enough metaphases for these experiments, we used lymphocytes isolated from both blood and spleen of each mouse. In preliminary experiments we confirmed that there was no difference in the radiation-induced dicentric frequencies in cells from these two tissues. Dicentric chromosome frequencies were assessed at days 7 and 60 after 0.4 Gy 350 MeV/n ^{28}Si ion irradiation or 1 Gy X-ray irradiation using three mice per time point. The data from the three mice were pooled together because no statistically significant differences between the mice were found. At least 500 metaphases were analyzed for each time/dose point.

Figure 8 shows that a dose of 0.4 Gy 350 MeV/n ^{28}Si -ion radiation is 2× less effective in dicentric chromosome induction compared to a dose of 1 Gy X rays. Another observation was that the numbers of dicentrics decreased from 0.15 per metaphase at day 7 to 0.03 dicentrics per metaphase at day 60 (or fivefold) for the X-ray-irradiated mice, and from 0.06 to 0.01 per metaphase in ^{28}Si -ion-irradiated mice (sixfold).

Chromosomal translocations assessed by mFISH—To detect chromosomal abnormalities with a focus on translocations in the early stages of human hematopoietic cell development, human CD45^+ , lin^- , CD34^+ , CD38^+ and CD45^+ , lin^- , CD34^+ , CD38^- cells were isolated from bone marrow via FACS sorting. Cells were obtained at days 7, 30 and 60 postirradiation. Matching our previous experiments, we used either a dose of 0.4 Gy of 350 MeV/n ^{28}Si ions or sham irradiation ($n = 3$ mice per condition). At least 100 metaphases per

condition were used in the analysis. Only one reciprocal translocation was scored out of 100 high quality metaphases at day 7 postirradiation. No aberrations were found in similarly processed specimens examined at days 30 and 60 postirradiation. No aberrations were seen in controls.

Histology Analysis of Bone Marrow Tissues from Controls and Irradiated Humanized Mice

Examination of the bone marrow of the ^{28}Si -ion-irradiated mice on days 7, 30 and 60 compared to the controls (see Supplementary Figs. S7 and Table S1; <http://dx.doi.org/10.1667/RR15148.1.S1>) revealed similar findings, consisting of cellular marrow (90–100% cellularity) with similar myeloid to erythroid ratio and progressive maturation.

Megakaryocytes were adequate in form and number in all samples, except in the day 7 ^{28}Si -ion-irradiated sample, which displayed focal clustering of small megakaryocytes. A solitary histiocytic cluster was seen in both a day 30 ^{28}Si -ion-irradiated sample and control sample. Hemosiderin deposition was noted in days 7 and 30 ^{28}Si -ion-irradiated sample, which was not identified in the control samples. In summary, these findings were all nonspecific, and likely reactive in nature, related to radiation-related changes.

DISCUSSION

Based on our experience, we believe that the humanized mouse model used in this work is essential for radiation studies. Advantages include the fact that radiation effects can be studied at every stage of human hematopoietic development in specific/distinct body systems/components of interest (e.g., bone marrow, spleen, peripheral blood, etc.) of the humanized mice *in vivo*. Human cell differentiation within the bone marrow of humanized mice resembles the human condition very closely, and both lineage-negative stages (involving the HSC and early progenitors) and lineage-positive stages (involving the myeloid and lymphoid lineages) can be studied in great detail. The model's characteristics are unmatched by any other existing system. An important aspect of this model is that it is based on transplantation of HSC purified from human cord blood. As such, properties and biological markers from aged individuals are underrepresented in the system. These properties include reduced repopulation capacity, increasing preference for myeloid over lymphoid differentiation, increasing of $\gamma\text{-H2AX}$ foci frequency (detected in individuals between 72–84 years of age) and reduced capacity to maintain HSC polarity (24–26). We therefore accept the possibility that a limitation of the HSC cells employed in this work may not recapitulate all of the characteristics of HSC from aged human individuals. Limitations of the model also include a murine peripheral blood distribution with a predominance of B cells and smaller fraction of T cells and a paucity of myeloid cells, as seen in most mouse strains (27). This is due to the fact that the bone marrow differentiation is driven by a set of mouse-specific growth factors and cytokines and, as a result, end stages of human myeloid blood cell development in the bone marrow are not completed. This drawback can be overcome by using existing NSG mouse strains engineered to express human SCF, GM-SCF and IL-3. However, such mouse strains need to be validated for radiation studies, since the presence of growth factors and cytokines may modify the radiation response of the engrafted human hematopoietic system. Another drawback is that there are large variations in the human cell counts in the mouse bone marrow, spleen and blood among the mice. Such

variations are not unusual even for subsets of human blood cell counts, for example, where the differences between healthy donors can vary 2–3-fold. What we found is that this large spread of data values presents a challenge in estimating the significance of modest radiation effects, but not when the effects are large. Importantly, the variation in cell count does not affect the developmental profiles in bone marrow and spleen. And finally, due to defective T-cell development, between 20–25% of the mice develop a graft-vs.-host disease (GVHD)-like condition approximately 5–7 months after successful human cell engraftment. This phenomenon is to some degree dependent on the HLA type of the human donor (28) and of the efficient depletion of CD3 cells from the cord blood-purified CD34⁺ cells.

The presence of GVHD lowers the numbers of mice that effectively can be used for long-term experiments, and we suggest that the experimental cohorts should be enlarged to compensate for these losses. Despite these limitations, the value of the model is still remarkable, thereby justifying additional effort and cost.

One of the challenges of human space travel is exposure to various kinds of particulate or electromagnetic radiation at energetic levels, which are exceedingly rare on Earth. A body of previously work has demonstrated a range of spaceflight-induced changes in human physiology, some of which concern radiation-induced perturbation of the hematopoietic system. Ancillary data from low-LET radiation studies indicate that, beginning with doses above 50 mSv, there are spill-over effects beyond this hematologic perturbation contributing to larger systematic effects, including infection, oncogenesis and mortality (29–31).

The nature of high-LET radiation damage in biological systems differs fundamentally from that of low-LET radiation. Specifically, high-charge and high-energy ions (HZE) impart a high fraction of complex DNA damage, i.e., localized clusters containing a mixture of single- and double-strand breaks (32, 33). As such, findings from studies using low-LET radiation cannot be directly applied to measure, for example, the probability of cancers induced by HZE nuclei in humans (34). Studies were undertaken to determine what pathologies were more directly attributable to high-LET radiation [reviewed in (35) and (36)]. Changes in peripheral blood cell counts, the impact of the mouse strain in radiation response, epigenetic alterations in bone marrow hematopoietic stem cells and other end points were reported (16, 37–42). Other studies show that high-LET radiation may induce tumors of the skin (43), mammary gland (44), liver and (23) and in intestines (45). Mouse models, however, are known to have issues with radiation response fidelity, complicating interpretation of comparative studies.

For example, the response of mouse and human hematopoietic stem cells to radiation is different. In mice, the response is biased toward the induction of pro-survival signaling pathways due to the activation of p53-dependent double-strand break repair. While the murine pro-survival bias imparts radioresistance and HSC protection, it also leads to accumulation of DNA damage (46). In contrast, human HSC exhibiting greater radiation sensitivity respond with bias toward apoptosis, sacrificing the damaged HSC population in favor of genomic integrity (47). The latter study also reports higher radiation sensitivity of human HSC compared to the rest of the human cell population in engrafted immunodeficient mouse human hematopoietic system. Our results are in accord with these reported trends.

We report significant short-term effects on the human HSC counts imparted by both high- and low-LET radiation exposure. Human HSC counts at day 7 after 350 MeV ^{28}Si irradiation were depleted fivefold, and this was sustained up to day 60 postirradiation, our last short-term time point. X rays induced an even larger HSC depletion at day 7 followed by a modest recovery 60 days postirradiation. Surprisingly, in response to both ^{28}Si ion and X-ray radiations, human CD45 counts in the bone marrow of the irradiated mice were equivalent to controls. A detailed comparative analysis of ten different cell populations representing different stages of human cell development returned no significant or persistent changes throughout the 60-day postirradiation period. These, we believe, are important results that point to a relative radiation resistance of human hematopoiesis in the bone marrow for the doses and energies we used. Simultaneously, these results indicate that even reduced HSC counts can maintain normal human hematopoietic cell differentiation in the mouse bone marrow.

A survey of changes in the spleen and peripheral blood after ^{28}Si -ion irradiation was performed using the same animals as in the bone marrow studies. In the spleen, a sustained threefold depletion of human CD45 cell counts was observed, which recovered to a 1.9-fold depletion at day 60 postirradiation. Subpopulations within the splenocyte pool showed no significant changes in cell type distributions at day 7 postirradiation, but a significant reduction in all B-cell subpopulations was observed at days 30 and 60 postirradiation. These findings are in contrast to the blood cell counts, in which a decrease of the cell counts was observed at only day 7 after ^{28}Si -ion irradiation.

X-ray-induced changes in splenocyte distributions differed from observed changes in comparable ^{28}Si -ion irradiations. While human cell numbers were greatly depressed at day 7 after X-ray irradiation, the population recovered to control levels by day 60 postirradiation. Subpopulation analysis in the X-ray-irradiated spleens showed an approximate twofold decrease of the B-cell populations at the day 7 time point, which normalized at day 60.

Thus, human CD45 splenocyte numbers are in contrast with the peripheral blood cell counts at day 60 after X-ray irradiation and this differs from the repopulation we observed after ^{28}Si -ion irradiation.

The long-term effects of ^{28}Si -ion radiation were studied in a large mouse cohort. Remarkably, there were no long-term effects of ^{28}Si ions on the human HSC counts, human bone marrow, spleen and blood cell counts. The bone marrow human developmental analysis also showed no differences between the irradiated and control mice. No long-term effects were studied in the bone marrow and spleen of X-ray-irradiated mice, but the blood cell counts evaluated at 4 months postirradiation showed a persistent effect and significant reduction of the human blood cell counts.

To conclude, exposure to 0.4 Gy 350 MeV/n ^{28}Si ions significantly depleted human HSC but did not perturb cell numbers or population distributions in the bone marrow. In both circulating cells and the splenocyte pool, ^{28}Si -ion irradiation was shown to transiently depress the human B-cell subpopulation to a significant extent (only at day 7 postirradiation for blood cells) before the population normalized to control levels. No sustained effects

attributable to ^{28}Si ions were observed in any of the studied hematopoietic compartments. Importantly, our clinical hematology panels returned no aberrant cell populations that would point to the commencement of an oncogenic or neoplastic process.

The *in vivo* effect of X-ray radiation on the human cells was different: X rays had very strong effect on the HSC at days 7 and 60 postirradiation (stronger than the effect of ^{28}Si ions), but no effect on the bone marrow human cell counts and bone marrow human cell development. X rays induced a decrease in human spleen cell counts and all B-cell subpopulations at day 7 postirradiation, but not at day 60. X-ray irradiation lead to persistent depression of blood cell counts at all studied short- and long-term time points. In addition, these findings indicate that the RBE of a dose of 0.4 Gy 350 MeV/n ^{28}Si radiation is lower than a dose of 1 Gy 250 kVp X rays for blood cell killing.

A possible explanation of these results is that HSC are very sensitive to high-LET Si ions but can maintain normal human cell counts in the bone marrow for all studied time points postirradiation. In addition, ^{28}Si ions has an effect on spleen function, which results in lower numbers of human cells that accumulate there. Assuming that the spleen functions as a depot of cells, but that this is not a controlling factor for the peripheral blood cell counts, we can reasonably account for these findings with such a model. These results show a complex short-term effect of ^{28}Si ion on the human hematopoietic system. Surprisingly, there were no long-term effects of ^{28}Si ion based on the studied parameters, despite the early effects that have been addressed above. This may indicate that the human HSC in this model have enough repair capacity to recover from the high-LET radiation. While this is supported by the observation that no aberrant populations were detected in the ^{28}Si -ion-irradiated mice, more data is needed to confirm such a conclusion.

In that respect, two other studies that investigated the effects of radiation on engrafted human hematopoietic system in humanized mice, support some of our conclusions. In one of the studies, humanized mice were used to investigate the effect of space mission-relevant doses of high-LET radiation on human hematopoietic cell reconstitution (48). For this study, human CD34⁺ cells were irradiated *in vitro* with 1 Gy of 50 MeV protons, 20 cGy of 1 GeV/n ^{56}Fe ions, 1 Gy of ^{137}Cs gamma radiation and a combination of both ^{56}Fe ions and gamma radiation. Cells were injected 18 h later into NSG mice and assessed for engraftment efficiency 5–9 months later. Interestingly, control animals exhibited the lowest overall engraftment and ^{56}Fe -irradiated animals showed the highest overall engraftment. While cell counts were not reported, terminally differentiated lymphocyte frequencies were reported and those values agree with our findings. The proportions of the different cell subpopulations in the mouse bone marrow and spleen were measured but the absolute cell counts that would illuminate the quantitative changes that might occur as a result of irradiation were not reported.

In the same article, the authors described a T-ALL development in the majority of a group of 20 cGy of ^{56}Fe -irradiated humanized mice. These results are different from those obtained in our current work. The discrepancy in findings could arise from the difference in experimental model. Specifically, we first engrafted the stem cells and then irradiated the

mice, whereas in the cited article, the authors first irradiated the stem cells and then injected the irradiated population into immunodeficient mice for engraftment.

In another article, Wang *et al.* reported on the effects of low-LET in a short-time course (12 weeks) after 0 and 1 Gy X-ray irradiations (49). Their findings of depressed human cell numbers in bone marrow and human HSC counts with reduced engraftment agreed with our findings.

Given the immediate, early and very pronounced effects of both radiation types on the HSC counts, we sought to check the cytogenetic status of the engrafted hematopoietic system. Radiation-induced dicentric chromosome formation in peripheral blood lymphocytes is considered the gold standard in radiation biodosimetry and is a commonly employed bioassay for assessment of accidental or occupational radiation exposure. Such data is time dependent and usually shows decreased dicentric frequencies over time, since cells with dicentrics cannot survive cell division. We studied the dicentric chromosome formation in human T cells isolated from mice at days 7 and 60 after *in vivo* ^{28}Si or X-ray irradiation. A significant increase in dicentric frequency was observed at day 7 postirradiation (6% dicentrics in the ^{28}Si -ion-irradiated mice and 15% in the X-ray-irradiated mice) followed by a rapid decrease of the dicentric frequencies measured at day 60 postirradiation. These results, we believe, are important, because the obtained data represent the systematic response to radiation for this end point. Interestingly, these findings show that 0.4 Gy of ^{28}Si ions is less efficient in dicentric induction than 1 Gy of X rays. A comparison of this *in vivo* data with *in vitro* data is difficult since there is a quantitative and qualitative difference in the measured chromosomal damage in these two conditions (34). Similar data obtained from blood cells of mice irradiated *in vivo* with 1 GeV/n of 1 Gy ^{56}Fe showed an average dicentric frequency of 8.72% at day 7 postirradiation. This frequency declined to 1.64% at day 60 postirradiation (50), a recovery that is similar to that observed in our system. In two important published studies, the chromosomal aberrations in the blood of astronauts after long-term missions were investigated (51, 52). In one study, data obtained from blood cells of 15 astronauts before and after orbital flights (duration of more than 3 months, average dose of 78 mGy) showed a statistically significant increase in the dicentric frequency with 0.4% greater relative to pre-flight blood samples. The dicentric frequency normalized to baseline when measured years later (51). In another study, no increase in dicentric frequency was observed in six astronauts when measured at 3–6 months after orbital flight (52). These studies are, of course, difficult to compare to our findings due to the large differences in dose and radiation quality. They do, however, support the notion that DNA repair mechanisms and/or apoptosis resolve the vast majority of radiation damage in the long term.

In addition to the dicentric analysis, we investigated ^{28}Si -ion-radiation-induced chromosomal translocations in lineage-negative CD34⁺ cells, which consist of HSC and progenitor cell fractions. These are rapidly dividing cell populations from which the hematopoietic system arises. We found no difference in the chromosomal translocation frequencies between irradiated and control cells at days 7, 30 and 60 postirradiation (only one chromosomal translocation was scored at day 7 in irradiated cells). The absence of translocations can be explained in two ways. It is possible that there was an increasingly small number of radiation-induced translocations that were undetectable in 100 scored

metaphases. Alternatively, the majority of cells with translocations died in the initial 7-day period postirradiation. We found no data in the published literature for chromosomal translocations derived from bone marrow lineage-negative cells for comparison. Results from assessment of chromosomal translocations in bone marrow cells isolated from *in vivo* irradiated mice show that the translocation frequency after 1 GeV/n ⁵⁶Fe-ion irradiation is in the range of 10%, while 1 Gy of gamma rays is in the range of 4%. Translocation frequencies were shown to be elevated in blood cells drawn from astronauts after spaceflight and decreased further over time (19, 52).

In summary, we have monitored the effect of high-LET and low-LET radiations on the entire process of human hematopoietic system development from bone marrow through the spleen and into peripheral blood. The combined results demonstrate that, in spite of early perturbation, the longer term effects of high-LET radiation are not detrimental to human hematopoiesis in our system of study.

The initial radiation-induced damage did not persist in our system of study. Also, the lack of tumor development shows that, at this energy and dose, high-LET exposure is unlikely to result in human hematopoietic cell transformation *in vivo* at least for the time period investigated here.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Effect of radiation on the human HSC (CD34⁺/CD38⁻). Panel A: Example of flow cytometry analysis of different cell populations in bone marrow of nonirradiated humanized mouse. Bone marrow mononuclear cells were stained with a panel of markers containing antibodies against human hCD45, lineage-positive markers, hCD34 and hCD38. The gate positions were estimated using fluorescence minus one (FMO) controls. Panels B-D show the frequencies of HSC in the lineage-negative cell population in bone marrow of humanized mice at different time points postirradiation. Panel B: HSC cells at days 7, 30 and 60 after 0.4 Gy 350 MeV/n ²⁸Si-ion irradiation; panel C: HSC 6–7 months postirradiation; panel D: HSC cells days 7 and 60 days after 1 Gy of 250 kVp X-ray irradiation. *HSC depletion is statistically significant ($P < 0.05$). Days 7 and 60 controls for the X-ray-irradiated mice (shown in gray) are combined in the graph.

FIG. 2.

Effect of radiation on the mouse and human CD45⁺ hematopoietic cell counts and human cell engraftment in the mouse bone marrow (panels A and C) and in the spleen (panels B and D) up to 60 days after 0.4 Gy of 350 MeV/n ²⁸Si-ion irradiation (panels A and B) and 1 Gy of 250 kVp X-ray irradiation (panels C and D). For 1 Gy X-ray irradiations, days 7 and 60 controls were pooled. Statistically significant differences between the irradiated and control mice are indicated (**P* < 0.05).

FIG. 3.

Effect of radiation on the mouse and human CD45⁺ hematopoietic cell counts and human cell engraftment in the bone marrow (panel A) and in the spleen (panel B) 6–7 months after 0.4 Gy of 350 MeV/n ²⁸Si irradiation. No statistically significant differences in the human cell counts were detected between the irradiated and control mice.

FIG. 4.

Effect of radiation on the human hematopoietic cell frequencies in the bone marrow of control and 0.4 Gy 350 MeV/n ²⁸Si-ion-irradiated mice at 6–7 months postirradiation. Comparison between the control and irradiated mice was performed using the *t* test, and no statistically significant differences were found for any of the cell subsets analyzed.

FIG. 5. Effect of radiation on the human hematopoietic cell frequencies in the spleens of control and 0.4 Gy 350 MeV/n ^{28}Si -ion-irradiated mice at 6–7 months postirradiation. Comparison of the control and irradiated mice was performed using the *t* test and no statistically significant differences were found for any of the cell subsets analyzed.

FIG. 6.

Effect of radiation on the depletion/recovery kinetics of human blood cell counts from humanized mice irradiated with 0.4 Gy of 350 MeV/n ^{28}Si ions (panels A-C) and 1 Gy of 250 kVp X rays (panels D-F), assessed at days 7, 30 and 60 postirradiation. Statistically significant changes in populations are indicated (* $P < 0.05$).

FIG. 7.

Effect of radiation on the mouse and human PBMC counts and human cell engraftment in blood of humanized mice irradiated with 0.4 Gy of 350 MeV/n ^{28}Si ions at 6–7 months postirradiation (panel A) and with 1 Gy of 250 kVp X rays at 4 months postirradiation (panel B). Statistically significant differences between irradiated and control cells are indicated (* $P < 0.05$).

FIG. 8.

Comparison of dicentric chromosome frequencies in human cells from humanized mice irradiated with 0.4 Gy of 350 MeV/n ²⁸Si ions and 1 Gy of 250 kVp X rays at days 7 and 60 postirradiation (n = 3 mice per condition). No dicentric chromosomes were detected in any of the controls.