Stem Cell Transfusion Restores Immune Function in Radiation-Induced Lymphopenic C57BL/6 Mice

Vaihali Kapoor1, Arpine Khudanyan1, Pilar de la Puente1, Jian Campian2,3, Dennis E. Hallahan1,2,4, Abdel Kareem Azab1,2, and Dinesh Thotala1,2

Abstract

Radiation-induced lymphopenia (RIL) is associated with treatment of different tumors (lung, colon, pancreas, breast, sarcomas, and glioblastoma). It is a significant clinical problem affecting the survival of cancer patients. The biologic mechanisms leading to RIL are not clearly understood. In this study, we established a mouse model of RIL representing therapeutic clinical regimen for lung cancer. Flow cytometry was used to analyze circulating levels of T and B cells and bone marrow (BM) stem cells. We found that fractionated radiation to the thorax significantly reduced circulating T and B cells as well as BM stem cells. Ex-vivo irradiation of blood and autologous reinfusion to mice also significantly induced lymphopenia. Furthermore, we found that mobilization of stem cells from the BM and autologous stem cell transplant rescued RIL in mice. Overall, our results suggest that RIL has not only direct effect on circulating lymphocytes, but also has indirect effect on circulating lymphocytes as well as stem cells in the non-irradiated BM. These results open a new window for investigating the direct and indirect biologic mechanisms leading to RIL, and provide a preclinical basis to test the effect of stem cell transplantation for treatment of RIL in cancer patients. Cancer Res; 75(17); 3442–5. ©2015 AACR.

Introduction

Radiotherapy is an integral part of lung cancer management (1). Despite significant advances in radiotherapy, normal tissue toxicity remains to be a significant side effect to patients undergoing radiotherapy. Radiotherapy was shown to decrease patients total lymphocyte counts to fall below 1,000/μL, a condition known as radiation-induced lymphopenia (RIL; ref. 2). RIL is frequently observed in cancer patients undergoing radiotherapy to various sites, including brain, thorax, abdomen, and pelvis (2–5). Recent clinical data indicate that RIL was associated with poor clinical outcome in patients with carcinomas of the lung, colon, pancreas, breast, sarcomas, and glioblastoma (2–4–6). The biologic mechanisms causing the development of RIL are not clearly understood. Given the extensive use of radiotherapy to treat cancer, there is a pressing need to understand the mechanisms involved in RIL, and to translate our understanding into novel therapeutic strategies to prevent and treat RIL.

In this study, we hypothesized that radiation not only has a direct effect on circulating lymphocytes, but also has an indirect effect on hematopoietic stem cells in the bone marrow (BM), which affects the development of T cells for prolonged periods.

Materials and Methods

Reagents and antibodies

AMD3100 (Sigma Aldrich), all antibodies for flow cytometry were from BD Biosciences. CountBrite beads were from Invitrogen.

Mice irradiations

All studies were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee and in accordance with protocols approved by the Washington University Division of Comparative Medicine. Six- to 8-week-old female C57BL/6 mice were obtained from Harlan laboratories. Mice were anesthetized with 2% isoflurane prior to partial body irradiations to the thorax by shielding other parts of the body with lead (2 Gy/day for 5 days). In some experiments, 200 μL of blood was drawn from mice, irradiated with one dose of 8 Gy ex vivo, and reinjected back to the respective mice. Mice and blood were irradiated using RS-2000 (Rad Source) irradiator at a dose rate of 1 Gy/min with 160 kVp X rays.

Mobilization of stem cells

AMD3100 (0 or 20 mg/Kg) was injected subcutaneously into mice and 4 hours later blood was drawn and peripheral blood mononuclear cells (PBMC) were harvested and cryopreserved. Peripheral blood was collected from each mouse before initiating any treatment and referred as baseline. PBMCs were injected to the mice after irradiation, and the levels of circulating lymphocytes were analyzed at different time points after injection.
Flow cytometry

PBMCs were isolated from mice blood after various treatments and stained with anti-CD3, CD4, CD8, CD19, and CD34 antibodies. BM mononuclear cells were also harvested from mouse femurs by flushing them with 1 mL of PBS and later stained with anti-CD34 antibodies. Cells were analyzed by MACSQuant Analyzer flow cytometer (Miltenyi Biotec) and data analyzed with FlowJo software (Tree Star Inc.). The cell counts were normalized to counting beads counts that were spiked in samples.

Figure 1.
Direct and indirect effects of irradiation on lymphopenia induction. A, diagram depicting the treatment plan in establishing the mouse model (C57BL/6) for RIL. Graph shows percentage of circulating CD3⁺ (T cells) and CD19⁺ (B cells) after 5 doses of 2 Gy irradiation to the thorax region at baseline (BL) and different days (1, 3, 14, and 21) after IR. SD is from four mice. B, diagram depicting the treatment plan to show indirect effects of thoracic irradiation on BM stem cells. Bar diagram shows percentages of CD34⁺ stem cells in the BM of mice that were either sham irradiated or irradiated with 2 Gy for 5 consecutive days to the thorax region. SD is from four mice. C, diagram depicting the treatment plan for ex-vivo irradiation experiment. Bar diagram shows percentages of circulating T and B cells before and after ex vivo IR and infusion. SD is from four mice. *, P < 0.05.

Figure 2.
Proposed model showing the biologic mechanisms involved in development of RIL.

Statistical analyses

All pair-wise comparisons between treatment groups were performed using the t test or ANOVA. A P value of <0.05 was considered statistically significant.

Results and Discussion

RIL is associated with treatment of many tumors (lung, colon, pancreas, breast, sarcomas, and glioblastoma) and associated with poor clinical outcomes (2, 3, 5, 6). The biologic mechanisms leading to RIL are not clearly understood.
T-cell renewal occurs approximately every 60 to 90 days (2, 5). If the effect of radiation was only on circulating lymphocytes, and assuming that all circulating T cells were destroyed by irradiation, lymphopenia should not be observed for more than 90 days. Prolonged RIL is however observed in patients for more than 6 to 12 months after irradiation (2). This led us to speculate that apart from circulating lymphocytes, radiation could also affect stem cells in the BM that primarily replenishes the lymphocytes. Furthermore, RIL was observed in patients even when radiation was administered to partial fields that did not include the BM (2). Therefore, we hypothesized that radiation has an indirect effect on the stem cells in the BM, leading to prolonged RIL.

To establish a mouse model of RIL representing lung cancer patients undergoing radiotherapy, we partially irradiated the mice in the thorax region with 2 Gy/day for 5 days (a total of 10 Gy). We then analyzed the number of circulating T cells (CD3⁺) and B cells (CD19⁺) in peripheral blood, as well as hematopoietic stem cells (CD34⁺) in the BM from the femurs, which were not directly exposed to irradiation, as shown in Fig. 1A and B. We found that the levels of T and B cells after the course of irradiation dropped to less than 5% of the baseline value before irradiation (Fig. 1A). These data from the mouse model are closest to RIL that is observed in lung cancer patients undergoing radiotherapy in the clinic. We used this mouse model of RIL to test our hypothesis that radiation has an indirect effect on the BM. We found that partial irradiation to the thorax region significantly reduced the population of CD34⁺ hematopoietic stem cells in the BM from the femurs that were not in the radiation field (Fig. 1B). These data support
our hypothesis and provide a direct evidence that irradiation has an indirect effect on the stem cells in the BM.

In addition, we hypothesized that similar to stem cells in the BM, irradiation may not only have a direct effect, but also an indirect effect on the survival of circulating lymphocytes. To test this hypothesis, we drew around 10% of the mouse blood (200 μL of approximately 2 mL total blood in the mouse), irradiated it ex vivo with 8 Gy, and injected the irradiated blood back to the respective mice. Twenty-four hours after irradiation/injection, we analyzed the number of circulating lymphocytes (Fig. 1C). Irradiating the blood with 8 Gy is sufficient to kill all the lymphocytes. If irradiation only induced direct cell death in lymphocytes, we should expect about 10% reduction in the lymphocytes population; however, we found that T cells and B cells decreased by 70% and 55%, respectively (Fig. 1C). These results indicate that irradiation of a small volume of the blood contains an indirect effect on circulating lymphocytes. Altogether, these results support our proposed model (Fig. 2) that irradiation induces lymphopenia through not only a direct effect on circulating lymphocytes, but also through indirect effect on circulating lymphocytes as well as an indirect effect on stem cells in the nonirradiated BM. Recently, induction of galectin-1 from cancer cells after irradiation has been shown to cause lymphopenia in mice (7). Similarly cytokines IL15 and IL7 were implicated to play a role in compensating lymphopenia induced by HIV infection and chemotherapy. However, IL15 and IL7 did not compensate lymphopenia induced by radiation (8). Therefore, more investigation is required to identify mechanisms involved in RIL.

Based on our observation that radiation reduces stem cell population in BM even if the BM was not directly in the radiation field, we hypothesized that autologous stem cell transplantation will rescue the mice from RIL. To test this hypothesis, we mobilized hematopoietic stem cells from the BM into circulation by administering AMD3100, a CXCR4 inhibitor (Fig. 3A). We found the levels of CD34+ cells, 4 hours post AMD3100 injection, increased 3-fold compared with base line levels (Fig. 3A). These results are similar to our previous observation that stem cells can be mobilized from the BM to the peripheral blood with the same kinetics in cancer models (9).

We collected PBMCs from untreated and AMD3100 treated mice and cryopreserved them. The mice were allowed to recover for 2 weeks prior to irradiating the thorax region with 2 Gy/day for 5 days to induce RIL (Fig. 1A). The cryopreserved PBMCs were thawed and autologously reinfused into the respective mice via tail vein (as described in Fig. 3B). We found that the mice that received stem cell–enriched PBMCs recovered faster from RIL than the mice that received just PBMCs. The levels of CD3+, CD4+, and CD8+ T cells, as well as CD19+ B cells recovered faster in the animals that received autologous stem cell transfusion (Fig. 3C).

Overall, these results strengthen our idea and open new opportunities to investigate the direct and indirect biologic mechanism(s) of radiation damage to lymphocytes and stem cells in the peripheral blood and in the BM. This could provide a preclinical basis for future clinical trials to test the effect of autologous stem cell transplantation for prevention of RIL in cancer patients.

Disclosure of Potential Conflicts of Interest
A.K. Azab reports receiving commercial research grant from Cell Works, Karyopharm, Selecyx, and Verastem and has ownership interest (including patents) in Targeted Therapeutics LLC. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: J. Campian, D.E. Hallahan, A.K. Azab, D. Thotala
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): V. Kapoor, A. Khudanyan, P. de la Puente, A.K. Azab, D. Thotala
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): V. Kapoor, A. Khudanyan, D.E. Hallahan, D. Thotala
Writing, review, and/or revision of the manuscript: V. Kapoor, J. Campian, D.E. Hallahan, A.K. Azab, D. Thotala
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.E. Hallahan, A.K. Azab
Study supervision: D.E. Hallahan, A.K. Azab, D. Thotala

Acknowledgments
The authors thank Rowan Karvas for help with the animal work.

Grant Support
This study was supported by Department of Radiation Oncology Startup Funds (D. Thotala).

Received May 22, 2015; accepted June 21, 2015; published OnlineFirst June 30, 2015.

References
Stem Cell Transfusion Restores Immune Function in Radiation-Induced Lymphopenic C57BL/6 Mice
Vaishali Kapoor, Arpine Khudanyan, Pilar de la Puente, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-15-1412

Cited articles
This article cites 8 articles, 2 of which you can access for free at:
http://cancerres.aacrjournals.org/content/75/17/3442.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.