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IL-12 Facilitates Both the Recovery of Endogenous Hematopoiesis and the Engraftment of Stem Cells after Ionizing Radiation

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Objective. **Severe myelosuppression is a common side effect of radiotherapy or chemotherapy. Methods have been developed to protect patients by stimulating white blood cell or red blood cell recovery/production using growth factors such as G-CSF or EPO. However, there is no available means to stimulate the full-lineage blood cell recovery from severe myelosuppression. In this study, we used lethally or sublethally irradiated animal models to evaluate the hematopoiesis stimulating effect of IL-12.**

Materials and Methods. **IL-12-treated lethally or sublethally irradiated animals were examined for the survival/lifespan, the function assays (bone marrow transplantation, CFU-S_{12}, CFC) of bone marrow cell subsets, and apoptosis assay.**

Results. **Using a low dose of IL-12 (10 times lower than previously reported dose), 91.4% of lethally irradiated animals survived long term without adverse effects on the gastrointestinal (GI) system. The reconstituted hematopoietic system was derived from long-term reconstituting hematopoietic stem cells (LTR HSC), which reconstituted hematopoiesis both endogenously after lethal radiation and in secondary recipients by bone marrow transplantation (BMT). IL-12 significantly attenuated the decline of blood cell counts in sublethally irradiated animals. The IL-12-stimulated hematopoiesis recovery resulted in a full-lineage blood cell production, including white and red blood cells, and platelets. There was no detectable expression of IL-12 receptor on LTR HSC. In IL-12-treated animals, the number of Sca-1^+ cells was significantly higher than in animals without IL-12 treatment.**

Conclusion. **In this study, we showed a low dose of IL-12 has hematopoietic-protecting effects, which can attenuate severe myelosuppresion caused by lethal or sublethal irradiation. This study, together with previous studies showing IL-12 is also an anti-tumor and anti-angiogenic agent, suggest IL-12 may have clinical significance in cancer treatment and BMT.** © **2007 International Society for Experimental Hematology. Published by Elsevier Inc.**

The hematopoietic stem cell compartment resides in the bone marrow and produces full-lineage blood cells throughout the lifespan. A functional hematopoietic system relies on HSC and their supporting microenvironment/niche. Via direct cell-cell interaction or soluble factors produced locally or systemically, the microenvironment regulates the quiescence, apoptosis, self-renewal, proliferation, and differentiation of stem cells [1–4]. The mechanism for this complicated regulation remains largely unknown. Accumulated evidence has shown that, as a niche component, osteoblasts play a pivotal role in HSC regulation [4–7]. Recently, using different approaches, two groups demonstrated that sinusoidal endothelial cells are another important element of the niche [8,9]. It has been shown that disruption of osteoblasts or sinusoidal endothelial cells results in the hematopoiesis dysfunction. On the other hand, stimulation of the osteoblasts or sinusoidal endothelial cells leads to increase of HSC numbers [6–8] or recovery of HSC from myelosuppession [9].

Bone marrow is the most sensitive organ to ionizing radiation and/or chemotherapeutic drugs during cancer therapy. Myelosuppression and hematopoietic dysfunction are the most common clinical complications following radio-/chemotherapy. These physical or chemical insults can

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damage hematopoiesis by targeting either HSC directly or alternatively their microenvironment or both. Obviously, it is important in cancer treatment to promote the recovery of hematopoiesis from myelosuppression. Consequently, a number of cytokines and combinations of cytokines have been studied using lethally irradiated animals [10–17]. Most of the studies observed the animal survival for 30 days after lethal radiation. With a combination of 5 anti-apoptotic factors, Herodin et al. reported 50% long-term survival (360 days) of the rescued animals. The receptors of the factors used in the previous studies, such as SCF, SDF-1, TPO, and Flt-3 ligand, are expressed on the HSC/progenitor cells. The protection from these factors may be a direct effect on the stem/progenitor cells. However, in the gastrointestinal (GI) system, the FGF-2-mediated radioprotective effect on intestinal crypt stem cells is via the protection of the endothelial cells adjacent to crypt stem cells, since FGF-2 receptor could only be detected on endothelial cells [18]. Since HSC function is closely related to the structure and function of bone marrow microenvironment, the recovery of hematopoiesis from lethal radiation requires the recovery of both the microenvironment and the HSC.

IL-12 is a heterodimeric pro-inflammatory cytokine that regulates the activity of cells involved in the immune response [19–21]. It stimulates the production of IFN-γ from natural killer cells and T cells, favors the differentiation of T helper 1 cells, and forms a link between innate resistance and adaptive immunity. Under in vitro conditions, IL-12 can stimulate hematopoiesis synergistically with IL-3 and SCF [22,23]. It has been reported that, at the price of sensitizing the GI system, IL-12 can protect the bone marrow from lethal irradiation [14]. However, in contrast to other radioprotective factors that may stimulate tumor cell proliferation or angiogenesis, such as SCF, SDF-1, and FGF-1 [24–27], IL-12 inhibits tumor cell growth [28] and is anti-angiogenic [29,30]. In this report, we explored the potential of using IL-12 to protect and facilitate hematopoiesis. We show that at a proper dose, IL-12 can protect the bone marrow hematopoietic system and promote engraftment in bone marrow transplantation (BMT) without adversely affecting the GI system. IL-12 may play its role by affecting the cells in the bone marrow microenvironment.

Materials and methods

Mice, cytokine, and antibodies

Six- to 8-week-old C57BL/6J female mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and maintained in the animal facility at University of Southern California (USC, Los Angeles, CA, USA) as described previously [31]. The animal study protocol was approved by the University of Southern California Animal Care and Use Committee. Recombinant murine interleukin 12 (rmIL-12) was purchased either from R&D Systems, Inc. (Minneapolis, MN, USA) or PeproTech Inc. (Rocky Hill, NJ, USA) and was dissolved in phosphate-buffered saline (PBS) at 100 ng/μL stock concentration according to the manufacturer's recommendation, and stored at −70°C. Antibodies used in the study were purchased from BD Biosciences (San Jose, CA, USA).

Radioprotection assay and blood cell count analysis

rmIL-12 was intravenously (i.v.) injected into mice before or after total-body irradiation at the indicated time points. Mice in the control group received PBS. Mice were lethally irradiated with 10 Gy given in two fractions of 5 Gy 3 hours apart or one dose of 10 Gy using dual, opposed sources of cesium 137 irradiator (Atomic Energy of Canada, Model: γ−cell 40).

In experiments to study the effects of IL-12 on lethally or sublethally irradiated animals (one dose of 10 Gy or 5 Gy), animals received either IL-12 (100 ng/mouse) or PBS at 24 hours before irradiation. Baseline of blood cell counts for each mouse was determined before IL-12 injection and then at different time points after irradiation. Because of the difference in absolute baseline blood cell counts between individual mice, we presented the data as the percentage change, thus normalizing the blood cell numbers of each time point to the numbers at baseline. To determine the peripheral blood cell counts, 10 uL blood was collected from the tail vein, diluted in 490 uL PBS buffer, and then analyzed in a MASCOT Multi-species Hematology System (CDC Technologies, Oxford, CT, USA).

Pathology of bone marrow, spleen, and small intestine

At different days after lethal dose irradiation (one dose of 10 Gy), femurs and small intestines were removed from IL-12-treated and control animals and fixed in 10% formalin buffer. The femurs were decalcified in Immunocal Formic Acid Bone Decalcifier (Decal Corporation, NY, USA) for 2 hours. The decalcified femurs and small intestine were embedded in TissuePrep 2 paraffin wax for micro-section at 5 μm and routine hematoxylin & eosin (HE) staining was performed. Slides were examined microscopically (Nikon E CLPSE E 8000, Camera: Diagnostic Model 2.2.1 with Spot RT software V3.5).

LTR HSC repopulation assay

To determine at what time point after IL-12 treatment and irradiation that the long-term reconstituting hematopoietic stem cells (LTR HSC) acquired the ability to rescue and repopulate other lethally irradiated animals, we did bone marrow transplantation using the cells isolated at different times postirradiation. After IL-12 administration (100 ng/mouse, 24 hrs before radiation) and irradiation (10 Gy, one dose), bone marrow cells from IL-12-treated or control (PBS-treated) mice (C57BL/Ly5.2 mice) were isolated at different times postirradiation (days 0, 1, 5, 7, 10, and 14 respectively). Bone marrow cells were flushed out and the red blood cells were lysed as previously described [31]. A total of 1×10^7 donor cells were transplanted to lethally irradiated recipients (C57BL/Ly5.1, 10 Gy, one dose) to determine the radiation rescue and long-term repopulation activity. Mice were observed daily and the long-term (>6 months) survival rate was recorded. There were 10 recipients for each time point.

We also determined if the bone marrow cells which repopulated in IL-12-protected mice long term can still repopulate other lethally irradiated animals. Six months after IL-12 treatment, bone marrow cells (C57BL/Ly5.2) were isolated and injected (1×10^6)