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Bone marrow production of lung cells: The impact of G-CSF, cardiotoxin, graded doses of irradiation, and subpopulation phenotype

Jason M. Aliotta[a], Patrick Keaney[a], Michael Passero[a], Mark S. Dooner[a], Jeffrey Pimentel[a], Deborah Greer[a], Delia Demers[a], Bethany Foster[a], Abigail Peterson[a], Gerri Dooner[a], Neil D. Theise[b], Mehrdad Abedi[a], Gerald A. Colvin[a], and Peter J. Quesenberry[a]

[a]*Roger Williams Medical Center, Center for Stem Cell Biology, Providence, RI, USA;* [b]*Department of Pathology and Department of Medicine, Division of Digestive Disease, Beth Israel Medical Center and Albert Einstein College of Medicine, New York, NY, USA*

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Objective. **Previous studies have demonstrated the production of various types of lung cells from marrow cells under diverse experimental conditions. Our aim was to identify some of the variables that influence conversion in the lung.**

Methods. **In separate experiments, mice received various doses of total-body irradiation followed by transplantation with whole bone marrow or various subpopulations of marrow cells ($Lin^{-/+}$, $c\text{-}kit^{-/+}$, $Sca\text{-}1^{-/+}$) from GFP^+ (C57BL/6-TgN[ACTbEGFP]1Osb) mice. Some were given intramuscular cardiotoxin and/or mobilized with granulocyte colony-stimulating factor (G-CSF).**

Results. **The production of pulmonary epithelial cells from engrafted bone marrow was established utilizing green fluorescent protein (GFP) antibody labeling to rule out autofluorescence and deconvolution microscopy to establish the colocaliztion of GFP and cytokeratin and the absence of CD45 in lung samples after transplantation. More donor-derived lung cells ($GFP^+/CD45^-$) were seen with increasing doses of radiation (5.43% of all lung cells, 1200 cGy). In the 900-cGy group, 61.43% of $GFP^+/CD45^-$ cells were also $cytokeratin^+$. Mobilization further increased $GFP^+/CD45^-$ cells to 7.88% in radiation-injured mice. Up to 1.67% of lung cells were $GFP^+/CD45^-$ in radiation-injured mice transplanted with Lin^-, $c\text{-}kit^+$, or $Sca\text{-}1^+$ marrow cells. Lin^+, $c\text{-}kit^-$, and $Sca\text{-}1^-$ subpopulations did not significantly engraft the lung.**

Conclusions. **We have established that marrow cells are capable of producing pulmonary epithelial cells and identified radiation dose and G-CSF mobilization as variables influencing the production of lung cells from marrow cells. Furthermore, the putative lung cell–producing marrow cell has the phenotype of a hematopoietic stem cell.**

Introduction

There is a growing body of evidence to suggest that adult hematopoietic marrow cells have much more versatile differentiation capabilities than once believed. Numerous studies have now demonstrated the ability of adult stem cells to differentiate into a variety of cells from nonhematopoietic organs, including the skin [1], muscle [2,3], bone [4], heart [5], brain [6], liver [7,8], and lung [9–13]. In murine models, structurally and functionally normal pulmonary epithelial cells, including bronchial epithelial cells [9] and type I (AE I) [10] and type II pneumocytes (AE II) [11], have been shown to be derived from exogenous marrow cells. These findings are exciting and of particular importance as a "normal" phenotype may be achieved in certain human pulmonary diseases (i.e., cystic fibrosis) if a relatively small number of normally functioning cells were to replace defective cells.

Injury is thought to be among the factors that influence the homing of marrow cells to the target organ and production of cells of the target organ. Intratracheal administration of bleomycin to the lung induces pulmonary fibrosis in

Offprint requests to: Dr. Jason M. Aliotta, M.D., Roger Williams Medical Center, Center for Stem Cell Biology, 825 Chalkstone Avenue, Providence, RI 02908; E-mail: jason.aliotta@verizon.net

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mice and has served as a valuable injury model. AE I are particularly sensitive to bleomycin and are the first alveolar cells to be injured, while AE II have a more variable sensitivity [14]. Kotton et al. [10] demonstrated the appearance of donor-derived cells in the lungs of mice as soon as one day after an infusion of *Lac*Z-labeled plastic-adherent cultured bone marrow cells following intratracheal bleomycin injury. Donor cells had the morphologic appearance and molecular phenotype of AE I. Other investigators have used radiation as a mode of lung injury. Alveolar epithelium damaged by radiation is repopulated as a result of AE II proliferation and conversion into AE I. Theise et al. [11] infused whole bone marrow cells into mice exposed to 1200 cGy of total-body irradiation (TBI). Within three days, their lungs appeared hypocellular, with breakdown of capillaries within alveolar septa and extravasation of erythrocytes into alveolar spaces. This effect peaked at day 5, at which time functioning, donor-derived AE II could be detected. Based on these and other studies, it would appear that injury is an essential catalyst that enables the production of lung cells from marrow; however, the degree of organ injury, the mode of injury used, and characteristics of the donor marrow cells may be among the multitude of factors that govern the extent of tissue replacement and the phenotype of newly produced cells.

We evaluated the role of a variety of factors in the production of lung cells from murine marrow cells. TBI was used not only to produce chimeric mice with exogenous marrow cells, but also as a mode of injury to the lung. In the present study, we show that higher doses of radiation lead to more blood cell chimerism, injury, and lung cell production.

Recombinant granulocyte colony-stimulating factor (G-CSF) is widely used to induce mobilization of blood precursors in different clinical settings such as chemotherapy-induced myelosuppression and peripheral blood stem cell recollection for autologous and allogeneic bone marrow transplantation [15]. It is the major growth factor responsible for regulating granulopoiesis and promoting the survival, proliferation, functional activation, and maturation of cells of the neutrophil lineage [15]. G-CSF has also been shown to increase the expression and function of adhesive receptors on the surface of both hematopoietic progenitors and stromal cells, including vascular cell adhesion molecule (VCAM-1) [15], potentially influencing the homing of marrow cells to certain tissues. We demonstrate here that G-CSF augments the homing of exogenous hematopoietic marrow cells to the radiation-injured mouse lung and production of lung cells.

Cardiotoxin, a component of cobra venom, causes an intense inflammatory reaction, resulting in muscle necrosis soon after direct injection. Its cytotoxic effect is thought to be mediated by its ability to competitively bind ATP, thus blocking the enzymatic activities of Na^+/K^+-ATPase [16]. Cardiotoxin is also believed to mediate inflammation by interacting with glycosaminoglycans (GAG), which are abundantly expressed in the tissues of the cardiovascular system [17]. GAGs function as co-receptors that facilitate the proper presentation of growth factors and chemokines necessary for cell migration, adhesion, and differentiation during wound healing and embryogenesis [17]. Cardiotoxin also directly injures the lungs as it has been shown to decrease the dynamic compliance of the lungs, induce hypoxemia by increasing the shunt fraction, and elevate pulmonary arterial pressures when injected into dogs [18]. It appears to induce these physiologic derangements by stimulating hemolysis and vascular congestion and increasing vascular permeability in the lungs by disrupting endothelial surfaces [19]. Our data indicate that this form of lung injury in irradiated mice has a minimal but statistically significant effect on the production of lung cells.

Cells with stem cell activity comprise only a very small percentage of the total number of cells present in unpurified whole bone marrow. Those with certain phenotypic characteristics, including the presence of stem cell antigen 1 (Sca-1) [20,21] and c-kit [21] or the absence of any cell markers (Lin^-) [20,21] that would define the cell as being differentiated, appear to be especially enriched with stem cell activity. We also demonstrate that hematopoietic marrow cells with these phenotypic characteristics preferentially reconstitute the bone marrow of radiation- and cardiotoxin-injured mice and produce lung cells.

Methods

Experimental animals

Six- to 8-week-old female C57BL/6 (H2Kb) mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and were used as bone marrow transplant (BMT) recipients. Animals were certified to be pathogen free, housed in our animal facility, and given ad libitum access to food and acid water. GFP-transgenic breeding pairs C57BL/6-TgN(ACTbEGFP)1Osb were also purchased from The Jackson Laboratory. Animals were bred in our animal facility by mating heterozygous GFP^+ mice, separated by using a UV light source, to C57BL/6 mice to produce GFP^+ transgenic animals. Our model is a β-actin promoter GFP transgenic. Homozygote animals die early. Other transgenics can be bred as homozygotes and we are evaluating these.

Isolation and preparation of whole bone marrow

Six- to 8-week-old female GFP^+ mice were used as donors. Mice were anesthetized with halothane and sacrificed by cervical dislocation. Whole bone marrow (WBM) was obtained by flushing the femurs, tibias, and pelvic bones with sterile 1× phosphate-buffered solution (PBS). Cells were centrifuged at 1300 rpm for 10 minutes at 4°C, then resuspended in sterile 1× PBS. Cells were counted after staining an aliquot with crystal violet.

Isolation and preparation of whole bone marrow for lineage depletion

WBM was isolated and prepared as described above. Cells were passed through a 25-gauge needle to make a single-cell suspension, then lineage depleted (Lin^-) by adding the following