Research Article

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From Bench Top to Bedside: Formation of Pulmonary Alveolar Epithelial Cells by Maintenance Cells and Healing Cells

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ABSTRACT

Pulmonary disease is a source of serous morbidity and mortality. Cell treatments offer hope for rejuvenation and repair of damaged lungs. The possibility of using maintenance cells and/or healing cells to repair damaged lungs has been studied for nearly two decades. This paper reviews pertinent research investigating the different models and approaches that have been studied concerning the use of donor-derived cells to increase alveolar stem cells in damaged lungs.

Keywords

Pulmonary alveolar epithelial cells, Maintenance cells, Healing cells, Bone marrow-derived stem cells, Side population cells, MAPCs, Hematopoietic stem cells, Non-hematopoietic bone marrow cells, Human cord blood, VSELs, MSCs, Lung progenitor cells, Induced lung injury, Irradiation, Chemotherapy, Asbestos, Detergent, Elastase, Endotoxin, Hyperoxia, Hypoxia, Lipopolysaccharide, Lysosomal acid lipase deficiency, Naphthalene, *Pseudomonas aeruginosa*, Type-II pneumocytes, Type-I pneumocytes, Murine, Mouse, Human, Cell culture, FISH-analysis, Y-chromosome analysis, Immunocytochemistry, FACS-analysis.

Introduction

There are three basic categories of cells within animals, i.e., functional cells, maintenance cells, and healing cells. The functional cells comprise the majority of the cell types and are composed of both stroma and parenchyma. They interact on a day-to-day basis with the animal's external and internal environments. A few examples of functional cells are type-I and type-II pneumocytes. Maintenance cells support the functional cells on a daily basis by replacing functional cells as they wear out and die as well as providing trophic factors for their function and survival. A few examples of maintenance cells are lung progenitor cells, multipotent adult progenitor cells (MAPCs), hematopoietic cells, and mesenchymal stem cells (MSCs). Healing cells are normally dormant and can be found hibernating within the stromal connective tissues throughout the body. Their function is to replace functional cells and maintenance cells lost due to trauma and/or disease. Examples of healing cells are bone marrow-derived stem cells, side population cells, and very small embryonic-like stem cells (VSELs) [1]. The studies reviewed will be discussed according to the type of experimental injury used to stimulate the formation of pulmonary alveolar cells from donor cells (Tables 1 and 2).

Radiation-Induced Injury Model

Krause et al. [2] found that administration of male bone marrow-derived stem cells (BMDSCs) led to the formation of 20% of the pneumocytes in the lung of female recipient mice who were irradiated from a dual-cesium source. They found that a single male donor marrow cell transplanted into lethally irradiated (1050-1100 cGy) female mice led to the formation of 20% of the pulmonary epithelial cells of the lung that were derived from the donor cell. Fluorescence in situ hybridization (FISH) for the Y chromosome and immunocytochemical (IMCC) staining for cytokeratins identified the donor-derived lung cells as epithelial lung cells, whereas FISH staining for the mRNA of surfactant protein B identified these cells as type II pneumocytes. Entire alveoli were lined with cytokeratin-stained marrow-derived

Authors	Type of Injury	Gender	Cell Type Transplanted	Mode of Administration	Effect Noted (if positive)
Krause et al. 2001 (2)	TBI: 1050-110 cGy	male donor, female recipient	10^7 Fr25lin¯ bone marrow cells labeled with PKH26	intravenous	type II epithelium 20%, GI tract epithelium 0.19-0.87%, skin epithelium 3.39%
Grove et al. 2002 (3)	TBI: 600 cGy	male donor, female recipient	2 x10^5 male GFP WBM	intravenous	Alveolar epithelium: 1-7% cytokeratin positive pneumocytes
Jiang et al. 2002 (4)	TBI: +/- 250 cGy; NOD/SCID	not specified	1 x 10^6 *ROSA26* MAPCS	intravenous tail vein injection	MAPCs differentiated hematopoietic lineage, and lung, gut, liver epithelium
Theise et al. 2002 (5)	TBI: 1200 cGy	male donor, female recipient	2 x 10^5 male WBM or 200 male CD34$^+$ lin¯cells	intravenous tail injection	pneumocytes 2-14% (WBM); type II pneumocytes 1.1-2.5% (CD34$^+$ lin¯cells)
Wagers et al. 2002 (6)	TBI (not specified)	Not specified	1 BM HSC	parabolic	single HSCs did not contribute appreciably to nonhematopoietic tissues (brain=1, liver=7, kidney=0, gut=0, lungs=0), no substantial chimerism noted in nonhematopoietic cells
Abe et al. 2003 (7)	TBI: 700, 950 cGy	male donor, female recipient	1-3 x 10^6 WBM or 2000 BM SP	Intravenous (side population cells)	Following BM mononuclear cell transplant: 37.9 ± 9.4% donor cells in lung; following BM SP cell transplant: 39.9 ± 9.3%
Abe et al. 2004 (9)	TBI: radiation ± elastase	not specified	BMDC	Parabiotic joining	lung fibroblasts (5-20%); type I alveolar cells, subepithelial fibroblast like interstitial cells, interstitial monocytes/ macrophages
Harris et al. 2004 (10)	TBI (not specified)	male donor, female recipient	BMDC	bone marrow transplant	at least 0.6% total lung cells EGFP-positive epithelial cells
Chang et al. 2005 (11)	TBI:5 Gy X 2 doses	not specified	WBM (2 x 10^7 cells)	transplanted bone marrow	No type II pneumocyte engraftment
MacPherson et al. 2005 (13)	TBI: 1050 cGY	Male donor, female recipient	1 x 10^4 cells ROSA26 SP bone marrow	intravenous tail injection	0.83% side population cell engraftment in tracheal epithelial lining; 55% Y-chromosome positive cells positive for cytokeratin epithelium marker
Kotton et al 2005 (14)	TBI:11 or 12 Gy single dose; 14 Gy (2 doses of 7 Gy)	not specified	10 x 10^6 BMC; 1 BM side population cell	intravenous (retro-orbital venous plexus)	None (no bone marrow derived epithelial cells)
Aliotta et al. 2006 (15)	TBI: 500, 900, or 1200 cGy	male donor, female recipient	5 x 10^6 cells WBM	intravenous tail injection	5.43% lung cells (900 cGy)
Bruscia et al. 2006 (16)	TBI: 400 cGY	male donor, female recipient	5-10 x 10^6 cells BMDSC	whole bone marrow transplant	epithelial engraftment with
Herzog et al. 2006 (17)	TBI: 400, 600, 1000 cGy	male donor, female recipient	2 x 10^6 cells WBM	bone marrow cells via tail vein injection	1-2% marrow-derived epithelial cells
Loi et al. 2006 (18)	TBI: 800 cGY	male donor, female recipient	2 x 10^6 cells BMC	intravenous tail injection	0.025% chimeric airway epithelial cells
Sueblinvong et al. 2008 (19)	TBI: 1400 cGY	non-obese diabetic/ severe combined immune-deficiency mice (gender not specified)	2 X 10^6 human umbilical cord blood-derived mesenchymal stem cells	intravenous tail vein injection	3.36% = total airway epithelial cells day 1; 0.72% 2 wk, 0.86% 1 month, 0.5% 3 months
Kassmer et al. 2012 (21)	TBI: 1000 cGy	male donor, female recipient	4.5-8 x 10^5 BMC (hematopoietic or non-hematopoietic)	retro-orbital plexus injection	0.08-1.8% type II pneumocytes (non-hematopoietic stem cell recipient)
Kassmer et al. 2013 (22)	TBI: 1000 cGy	not specified	900-1500 cells: very small embryologic like cells (VSELs); 100,000 non-VESL cells	retro-orbital plexus injection	4% type II pneumocytes (VSELS transplant); 0.36% type II pneumocytes (non-VSELS)
Kotton et al. 2001 (8)	Bleomycin (BLM) (intra-tracheal)	female donor, female recipient	1-2 x 10^6 MSC	intravenous tail injection	type I pneumocytes (not quantified)
Ortiz et al. 2003 (24)	Bleomycin (intra-tracheal)	male donor, female recipient	5 x 10^5 MSC	Intravenous	29% lung cells (<5% in bleomycin treat intact marrow animals); Bleomycin (BLM) exposure increased engraftment of mesenchymal stem cells (MSC) 23 fold; MSC administration after BLM also decreased inflammation and collagen deposition
Hashimoto et al. 2004 (25)	Bleomycin (intra-tracheal); TBI: 1000 cGy	Female mice	4 x 10^6 WBM	retro-orbital injection under anesthesia for BM chimera mice	71% BM-derived cells in BLM-treated chimera mice vs 17% in controls; <80% of Col+ cells were GFP+ in BLM-treated GFP BM chimera mice (represents 27.5% of whole-lung cells analyzed)
Rojas et al. 2005 (26)	Bleomycin ± busulfan	male donors, recipient not specified	5 x 10^6 cells/ml suspension BMDMSC	intravenous tail injection	~29% lung cells (BLM after myelosuppression); <5% (BLM treated with intact bone marrow)
Ortiz et al. 2007(27)	Bleomycin	male donor, female recipient	5 x 10^5 cells MSC	intravenous (jugular)	0.54% and 0.01% donor lung cells at days 7 and 14; MSCs engraftment elevated by BLM exposure, Il1RN not as effective as MSCs in modulating inflammatory response to BLM
Moodley et al. 2009 (28)	Bleomycin (intranasal)	not specified	1 x 10^6 uMSC or 1 x10^6 fibroblasts	intravenous tail injection	uMSCs reduced number of infiltrates and number of fibrotic foci at 7, 14, and 28 days

Reese et al. 2008 (29)	BCNU; BG	not specified	2 x 10^6 5-FU WBMC or 1000 SKL cells supported with 2-4 X10^6 cells	whole bone marrow transplant	Donor cells in absence of BCNU injury at 3 months 16%; untreated mice at 3 months 20.3%; after BG and BCNU treatment 25%
Hashimoto et al. 2004 (25)	Bleomycin (intratracheal); TBI: 1000 cGy	Female mice	4 x 10^6 WBM	retro-orbital injection under anesthesia for BM chimera mice	71% BM-derived cells in BLM-treated chimera mice vs 17% in controls; <80% of Col+ cells were GFP+ in BLM-treated GFP BM chimera mice (represents 27.5% of whole-lung cells analyzed)
Rojas et al. 2005 (26)	Bleomycin ± busulfan	male donors, recipient not specified	5 x 10^6 cells/ml suspension BMDMSC	intravenous tail injection	~29% lung cells (BLM after myelosup-pression); <5% (BLM treated with intact bone marrow)
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Reese et al. 2008 (29)	BCNU; BG	not specified	2 x 10^6 5-FU WBMC or 1000 SKL cells supported with 2-4 X10^6 cells	whole bone marrow transplant	Donor cells in absence of BCNU injury at 3 months 16%; untreated mice at 3 months 20.3%; after BG and BCNU treatment 25%
Beckett et al. 2005 (34)	Endotoxin induced	Male donor, female recipient	20-40 x 10^6 MSC	bone marrow transplant	majority of marrow-derived cells recruited to lungs were CD45^{-}positive leukocytes
Gupta et al. 2007 (35)	Endotoxin (lipopolysaccharide intra-tracheal)	Male mice	MSC (750,000)	intratracheal	<5% engraftment at 48hr post injury; increased survival/repair & decreased inflammation/edema but MSC administration in endotoxin-induced model reduced injury
Xu et al. 2007 (36)	Endotoxin (intra-peritoneal lipopolysac-charide)	Female Mice	5 x 10^5 cells BMDMSC or fibroblast	intravenous	BMDMCS suppression of endotoxin-induced local/system inflammation; No BMDMSC lung engraftment or differentiation of stem cells
Fritzell et al. 2009 (37)	Hyperoxia treated	Not specified	10 x 10^6 BMCGTP^{+} cells	intranasal	No epithelial reconstitution achieved
Yamada et al. 2004 (38)	Lipopolysaccharide	Male mice	whole bone marrow/fetal liver cells (2 x 10^6 cells)	intravenous through tail vein	BMPC required for lung repair following LPS-induced injury
Zhang et al. 2014 (39)	Lipopoly-saccharide, passive smoking	male donor, female recipient	4 x 10^6 MSC	intravenous tail vein injection	MSC transplantation promote collagen fiber deposition and play role in lung repair
Yan et al. 2007 (40)	Lysosomal acid lipase	Not specified	(1-2 x 10^7 cell/300ųl BMSC or 1 X 10^6 cell/100ųl	tail vein injection	10-18% alveolar type II epithelial cells (8 wks)
Loi et al. 2006 (18)	napthalene-induced airway	Male donor, female recipient	total marrow (20 x 10^6 cells)	intravenous tail injection	Non-injured animals: 0.33%, naphthalene 1 week: 1.0%, uninjured 1 month: 0.5%, naphthalene 1 month: 1.5%, uninjured 3 months: 2%, naphthalene 3 month: 5%
Serikov et al. 2007 (41)	napthalene-induced airway	Male donor mice, female recipient	1 x 10^6 MSC	intratracheal	partial engraftment of MSC to bone marrow 1 month posttransplant; GFP+ cells in lung parenchyma and airway epithelium at 2-6 days post naphthalene; only very rare GFP+ cells patches in epithelium at day 30
Wong et al. 2007 (42)	napthalene-induced airway	Male donor mice, female recipient	1 x 10^6 cells BMC	intravenous and intratracheal	day 2: 2-5%; day 14: 3.35% (at most 1 % after complete airway epithelial regeneration)
Giangreco et al. 2009 (43)	napthalene-induced airway	11 chimeric males, 2 females	NEB- and BADJ-associated stem cells (i.e. variant CCSP-expressing cells)	chimera mouse model	stems cells don't contribute to lung homeostasis; activation contingent upon epithelial progenitor cell depletion post lung injury
Wong et al. 2009 (44)	napthalene-induced airway	Male donor mice, female recipient	1 x 10^6 cells BMC	intravenous and intratracheal	population of bone marrow cells that expressed CCSP (1.9%) expanded in culture up to 25.8% after 7 days
Rejman 2009 (45)	Pseudomonas aeruginosa (10^5 and 10^6 cfu)	male donor, female recipient	10 x 10^7 cells: intravenous, 0.5-1 x 10^6: intratracheal)	intravenous and intratracheal	0.6% and 1.12% GFP +cells expressing CK for 10^5 and 10^6 cfu bacterial inoculums

Table 1: Mouse Research.

Authors	Type of Injury	Gender	Cell type Transplanted	Mode of Administration	Effect Noted (if positive)
Kleeberger et al. 2003 (46)	Human Sex Mismatched Lung Transplants	not specified	bone marrow derived precursors	transplant	bronchial epithelium (6-26%), type II pneumocytes (9-20%) and seromucous glands (9-24%).
Suratt et al. 2003 (47)	Human Sex Mismatched Lung Transplants	male donors, female recipient	related allogenic bone marrow and peripheral blood stem cell; unrelated allogeneic cord blood transplant	transplant	significant rates of epithelial (2.5-8%) and endothelial (37.5-42.3%) chimerism
Mattson et al. 2004 (48)	Human Sex Mismatched Lung Transplants	female recipients, male donors	Hematopoietic stem cell (HSC)	allogeneic hematopoietic stem-cell transplant	complete donor chimerism, detection of Y-chromosome-positive epithelial lung cells (2% and 6%) and surfactant-positive male epithelial cells

Mattson et al. 2004 (48)	Human Sex Mismatched Lung Transplants	female recipients, male donors	Hematopoietic stem cell (HSC)	allogeneic hematopoietic stem-cell transplant	complete donor chimerism, detection of Y-chromosome-positive epithelial lung cells (2% and 6%) and surfactant-positive male epithelial cells
Zander et al. 2006 (51)	Human Sex Mismatched Lung Transplants	male donors, female recipient	hematopoietic cells (peripheral blood or marrow)	transplanted mobilized peripheral blood or bone marrow	1.75% type II pneumocytes
Spees et al. 2003 (52)	heat shocked cells in culture	male and female donor cells	2.5-5 X 10^5 cells GFP$^+$ hMSC	ex-vivo model derived from bone marrow aspirates	GFP+ hMSCs differentiated into bronchial epithelium-like cells with heat shock, up to 1% cell fusion
Wang et al. 2005 (53)	Cystic fibrosis (co-culture)	CF patients, sex not specified	MSC expressing GFP (1 x 10^6 cells/ml)	ex vivo model	CFTR expression in MCSs after co-culture with airway epithelial cells; MSCs differentiation into airway epithelium

Table 2: Human Studies.

epithelia, demonstrating that the type II pneumocytes had given rise to type I pneumocytes as well. Donor-derived epithelial cells were also detected in the gastrointestinal tract (0.19%-0.87%) and skin (3.39%). The use of cytokeratin staining and IMCC staining for C11b allowed the epithelial cells to be distinguished from hematopoietic cells such as macrophages, lymphocytes, and polymorphonuclear leukocytes. Type II pneumocytes were detected as early as 5 days after transplant. These authors noted that two patterns of epithelial engraftment were present in their work. There was a large-scale repopulation in response to injury (in the damaged liver and lung) and low-level engraftment as individual scattered cells in the absence of marked injury (in the liver, skin and gastrointestinal tract). The authors hypothesized that the high percentage of Y chromosome-positive pneumocytes may reflect an early proliferative healing response to acute radiation injury and possible post-radiation infection.

In another paper from Krause's group, Grove et al. [3] used irradiated (600 cGy on 2 consecutive days) female mice that were transplanted with male marrow (0.2 million cells) that had been transduced with retrovirus encoding green fluorescent protein expression (eGFP). Transgene-expressing lung epithelial cells were present in all recipients analyzed at 2, 5, or 11 months after transplantation, demonstrating that highly plastic bone marrow stem cells (BMSC) can be stably transduced in vitro and retain their ability to differentiate into lung epithelium while maintaining long-term transgene expression. Approximately one tenth of all eGFP-positive cells in the lung were identified as cytokeratin-positive pneumocytes; these cells comprised an average of 3% of all alveolar cells in the lung tissue of BMT recipient mice.

Jiang et al. [4] studied the ability of murine multipotent adult progenitor cells (MAPCs) to engraft to form pulmonary alveolar epithelial cells. To avoid rejection by recipient animals, 1 million undifferentiated *ROSA26* MAPCs were injected via tail vein into 6-8 week-old irradiated (250 cGy) or non-irradiated non-obese diabetic/severe combined immunodeficient (NOD-SCID) recipients.

In the lung, approximately 4% of pan-CK+ CD45- alveolar cells were β-gal+. Morphologically, multipotent adult progenitor cells (MAPCs) are 8-10 μm in diameter with a large nucleus and scant cytoplasm. Cultured murine multipotent adult progenitor cells (mMAPCs) are CD34- CD44- CD45- c-Kit- and negative for MHC class I and II. They express low levels of Flk-1, Sca-1 and Thy-1, and higher levels of CD13 and SSEA-1.

In another paper from Krause's group, Theise et al. [5] utilized female mice irradiated with 1200 cGy. They received whole bone marrow transplants of 20,000 cells from age-matched male donors. The females were sacrificed at 1, 3, 5 and 7 days and 2, 4 and 6 months after transplantation. Irradiation caused histologic evidence of pneumonitis including alveolar breakdown and hemorrhage beginning at day 3. Male-derived pneumocytes were identified by simultaneous fluorescent in situ hybridization (FISH) for the Y chromosome and mRNA for surfactant B on lung tissue. Y-positive pneumocytes were engrafted as early as day 5. Eventually 2-14% of pneumocytes were derived from male donors. By two months, marrow-derived pneumocytes could comprise entire alveoli, suggesting that type I pneumocytes was derived from type II pneumocytes. In addition, two female mice received 200 male fluorescence-activated cell sorted CD34+ lin- cells. These females were sacrificed at 8 months after transplantation. Donor-derived type II pneumocytes ranged from 1.1-2.5% in these two females.

Wagers et al. [6] transplanted mouse green fluorescent protein-labeled bone marrow-derived hematopoietic stem cells (BMD HSC) into lethally irradiated mice. Single HSCs did not contribute cells to the lung, kidney, gut or muscle at 4 to 9 months after transplantation. The lung cells did not stain for the epithelium-specific marker pan-cytokeratin. HSCs engrafted into hematopoietic but not non-hematopoietic cells. 18% of cells of hematopoietic lineage demonstrated chimerism. The vast majority of labeled cells displayed the CD45 pan-hematopoietic marker CD45. Wagers et al. [6] using parabiotic mice found that steady-state conditions, progenitor cells from the untreated partner were found to be green fluorescent protein-positive. However, after irradiation, GFP positive cells were found in the lung of the untreated mouse, indicating that they were derived from parabiotic partners.

Abe et al. [7] investigated whether 1-3 million purified mononuclear bone marrow (BM) cells or 2,000 side population (SP) cells isolated by fluorescence activated cell sorting (FACS) could give rise to progeny in the lungs, and whether total body irradiation (TBI) was necessary. Transgenic green fluorescent protein-expressing (eGFP) donors were used to isolate the bone

marrow (BM) and side population (SP) cells. The lungs were evaluated using flow cytometric evaluation of cell suspensions, or immunocytochemical (IMCC) detection of green fluorescent protein (GFP)-positive cells. Experimental mice received a mid-lethal dose (7 Gy) followed by a lethal dose (9.5 Gy) of radiation. 30-140 days after stem cell transplantation, 37.9 ± 9.4% bright green donor cells were found in cells suspensions of the lungs of recipients when 1 million BM-derived mononuclear cells were transplanted. Transplantation of 2000 BM SP cells produced 39.9 ± 9.3%. However, when 2 million donor-derived BM cells were transplanted into non-irradiated (intact) recipients, no donor-derived cells could be detected in blood or lung 30 days after transplantation. Morphologically, donor-derived cells in the lung were primarily monocytes/macrophages and potentially intra-epithelial lymphocytes. Fibroblast-like cells, and type I (but not type II) alveolar epithelial cells were observed. Thus, this study agrees with the work of Kotton et al. [8], in that the epithelial alveolar cells were type I rather than type II.

Abe et al. [9] showed that irradiation or radiation with elastase treatment significantly increased the proportion of cells derived from bone marrow in a parabiotic experiment. 5-20% of fibroblasts were derived from the circulating blood. Interstitial monocytes/macrophages, subepithelial fibroblast-like interstitial cells, and type 1 alveolar epithelial cells were identified as being derived from bone marrow cells.

Harris et al. [10] used the Cre/lox system together with β-galactosidase and enhanced green fluorescent protein expression in transgenic mice to identify epithelial cells. In the lung, at least 0.6% of the total lung cells were EGFP-positive epithelial cells.

Chang et al. [11] used donor bone marrow harvested from transgenic mice expressing the LacZ or eGFP gene ubiquitously, or under the control of the human surfactant protein C (SP)-C promoter. The bone marrow was transplanted into lethally irradiated neonatal mice. In recipients transplanted with marrow that expressed lacZ or eGFP ubiquitously, light microscopy revealed cells whose morphology and location were compatible with a type II alveolar epithelial cell phenotype. Co-localization by fluorescence microscopy eGFP and pro-SP-C proteins in single cells was noted. However, further analysis revealed that the putative type II pneumocytes were actually artifacts due to overlapping fluorescent signals between cells positive for green fluorescent protein (GFP) and those positive for surfactant protein C. These results underscored the technical difficulties associated with evaluating engraftment in the lung, and argued against a contributory role for marrow cells in populating the alveolar epithelium after injury. This study has been discussed by Krause [12]. She noted the rigorous techniques used by Chang et al. [11], but noted that the GFP donor mice used by these authors had strong but variegated expression of GFP in adult liver, kidney, small intestine and lung. Furthermore, the recipient mice were 3 days old at the time of transplantation and donor cells were administered by intraperitoneal injection.

MacPherson et al. [13] examined the airway tissue of lethally-irradiated female mice that were rescued by injection of male SP *Rosa26* bone marrow-derived cells. Their studies utilized female mice (8-12 weeks of age) that were lethally irradiated with 1050 rads. Following irradiation they received 10,000 bone marrow-derived side population (SP) cells via tail vein. They were housed for 3 months, and then the tracheas were damaged with by instillation with 10 microliters of 2% polidocanol and the tissues harvested 7 days later. MacPherson et al. [13] noted that their Isolated SP cells have a high nucleus/cytoplasm ratio. Tissue analysis was performed by FISH analysis for the Y chromosome and cytokeratin staining by immunocytochemistry. The polidocanol treatment completely stripped the epithelial layer of the tracheas, but the epithelial lining had been replaced after 7 days. The average number of donor-derived cells in the trachea was 0.83% (range 0.45-11.5%). 55% of the Y chromosome positive donor cells were found to be positive for the cytokeratin epithelial marker. It should be noted that Krause et al. [2] found few cells in the trachea compared with the lung.

Kotton et al. [14] reported that hematopoietic stem cells (HSCs) transplanted into lethally irradiated mice. The mice were lethally irradiated with either 11 Gy or 12 Gy of radiation in a single dose or 14 Gy as two doses given 3 hours apart on the day before transplantation. 200 donor hematopoietic stem cells obtained from surfactant protein C-GFP or β-actin transgenic mice mixed with 200,000 unfractionated CD45.1 competitor bone marrow cells were intravenously injected retro-orbitally into recipient mice. More than 99 percent of the cells that stained with pro-SP-C, a marker for AT type II alveolar epithelial cells, did not exhibit fluorescence for GFP. More than 99% of the cells in the lung that were GFP-positive did not stain for pro-SP-C. 1.39 cells per tissue section exhibited staining for both GFP and pro-SP-C. However, this was not different from background staining.

Aliotta et al. [15] studied the effects of whole body irradiation on the production of lung cells from bone marrow cells. Following specific doses of whole-body irradiation, mice received transplants of whole bone marrow or various subpopulations of marrow cells. Criteria for the presence of bone marrow-derived epithelial cells were as follows: (1) green fluorescent protein antibody labeling; (2) cytokeratin antibody labeling; absence of CD45 in lung samples after transplantation. Increasing doses of radiation led to increases in bone marrow-derived epithelial cells in the lungs receiving the transplants. Thus 900 cGy of radiation led to labeling in 5.43% of all lung cells.

Bruscia et al. [16] tested whether transplantation of bone marrow-derived stem cells into one day-old mice would result in higher levels of engraftment than that seen in adult mice. Donor-derived epithelial cells were found. The highest epithelial engraftment (0.02%) was obtained in mice that received a preparative regimen of busulfan in utero. However, bone marrow transplantation into newborn mice, regardless of the myeloablative regimen used, did not increase the number of bone marrow-derived epithelial cells over that which occurs after bone marrow transplant into lethally-irradiated mice. 5-10 million cells were injected into the temporal

vein. The Y chromosome was determined by fluorescence in situ hybridization (FISH) and immunofluorescence for different cell type-specific markers was performed. Several different treatment regimens were employed. Regimen 1 involved two doses of busulfan (an antineoplastic alkylating agent). Regimen 2 consisted of one dose of busulfan, followed by 400 cGy of total body irradiation. Regimen 3 consisted of 400 cGy of total body irradiation (TBI). Regimen 4 involved 400 cGy of TBI. Eighty to 100 days after bone marrow transplantation, the GI tract, lungs, and liver of transplanted mice were analyzed for bone marrow-derived epithelial cells. Lungs were also analyzed by cytokeratin immunofluorescence. Without any myelosuppression, donor-derived epithelial cells were detected in one of four mice at the rate of 0.003%. The largest amount of donor-derived epithelial cells in the lung was 0.02% in the regimen 1 group. The busulfan/400 cGy TBI group (regimen 2) exhibited 0.009%, and the sublethally-irradiated 400 cGy group (regimen 4) exhibited no engraftment. Thus the rate of formation of donor-derived epithelial cells in one day-old mice was no greater than that observed with adult rats.

Herzog et al. [17] used sex-mismatched bone marrow transplantation to study the threshold of damage required to produce engraftment of stem cells to form pulmonary epithelial cells. Female mice were exposed to 400, 500 or 1,000 cGY. Six hours later the female mice received 2 million male whole bone marrow cells via tail vein injection. Varying doses of irradiation did not correlate with the presence of donor-derived epithelia in the recipient lung. Bone marrow derived epithelial cells were found in the lungs only among the mice that received the highest dosage of radiation. Their morphology and location was consistent with type II pneumocytes. Contrary to an earlier report from the same laboratory that found 15-20% of BMD epithelial cells (1), Herzog et al. [17] found 1-2% of the cells were bone-marrow derived epithelial cells. They postulated that induction of lung damage may be required for BMD cells to engraft as pneumocytes.

Loi et al. [18] transplanted 20 million bone marrow cells from male wild-type mice into the tail vein of adult female Cftr knockout mice that had been subjected to total body irradiation (800 rads) using a cesium irradiator. Donor-derived cells were assessed by fluorescence in situ hybridization (FISH) analysis for detection of cells positive for the Y chromosome. Examination of the recipient lungs demonstrated rare (0.025%) chimeric airway epithelial cells, some of which expressed cystic fibrosis transmembrane conductance regulator protein (CFTR). Most of the cells were located in the alveolar walls. Most were CD45 negative (and therefore not leukocytes). They had the morphologic appearance of alveolar epithelial and/or endothelial cells.

Sueblinvong et al. [19] administered human cord blood mesenchymal stem cells (CB-MSCs) to immunotolerant, non-obese diabetic/severe combined immunodeficient (NOD-SCID) mice. Their lungs were analyzed for the presence of human cells. After systemic administration via the tail vein to immunotolerant NOD-SCID mice, up to 3.36% of the total airway epithelial cells counted 1 day after CB-MSC administration were derived from these human (human β2-microglobulin staining) cells. This dropped to 0.72% by 2 weeks, 0.86% at 1 month, and 0.5% at 3 months. When adjusted for anatomical location and morphology, up to 0.25% of the CK-positive airway epithelial cells were dual labeled (with β2 -globulin and cytokeratin). Sueblinvong et al. [19] concluded that CB-MSCs can express phenotypic markers of airway epithelium and can participate in airway remodeling in vivo.

Kassmer and Krause [20] critically reviewed the literature involving whether bone marrow-derived cells can form pulmonary epithelial cells. They worked to establish criteria needed for detection of these cells. They postulated that the following are mechanisms by which marrow derived cells can lead to the appearance of pulmonary epithelial cells. (1) Transdifferentiation-or plasticity; (2) differentiation of a precursor; and (3) fusion followed by reprogramming. "Transdifferentiation" or plasticity was defined as one committed cell becoming another type of committed cell, and they note that has not been definitively proven to date. They postulate that mechanisms by which marrow derived cells can lead to the appearance of pulmonary epithelial cells could involve more than one of these mechanisms.

Kassmer et al. [21] transplanted either wild type hematopoietic or non-hematopoietic bone marrow cells into irradiated surfactant-protein-C (SPC)-null mice. Donor-derived SPC-positive type 2 pneumocytes were predominantly detected in the lungs of mice receiving purified non-hematopoietic stem and progenitor cells. They concluded that cells in the non-hematopoietic fraction of the bone marrow are the primary source of marrow-derived lung epithelial cells. They hypothesized that these non-hematopoietic cells may represent a primitive stem cell population residing in adult bone marrow.

Kassmer et al. [22] demonstrated that non-hematopoietic bone marrow cells are the primary source of bone marrow-derived epithelial cells of the lung. These studies challenge the view that adult stem cells have a restricted potential, and suggest that adult stem cells from one tissue are able to give rise to cells of a different lineage, a phenomenon termed "plasticity." Kassmer et al. [22] tested in mice the hypothesis that very small embryonic-like stem cells (VSELS) are responsible for engraftment to form epithelial cells of the lung. They directly compared the level of bone marrow-derived (BM derived) epithelial cells following transplantation of either VSELS, hematopoietic stem/progenitor cells, or other non-hematopoietic cells. Transplantation with 900-1500 VSELS resulted in formation of 4% of all T2 pneumocytes. By contrast, transplantation with 100,000 non VSEL stem cells (from the non-hematopoietic bone marrow fraction) resulted in formation of only 0.36% of T2 pneumocytes were found in the lungs of recipient mice. These transplantations consistently gave rise to surfactant-positive epithelial cells in SPC-knockout mice. Kassmer et al. [22] demonstrated that VSELS from the bone marrow differentiate into epithelial cells of the lung, and that this process is independent of cell fusion. They noted that VSELs have the highest rate of forming epithelial cells in the lung of all the cell types in bone

marrow. Tissue damage is necessary for the appearance of bone marrow-derived epithelial cells in the lung [12,23].

Bleomycin-Induced Injury Model

Kotton et al. [8] intravenously administered Lac-Z labeled plastic adherent cultured murine bone marrow cells into wild-type mice that had received lung injury with bleomycin. Marrow derived cells engrafted in recipient lung parenchyma as cells with the morphological and molecular phenotype of type I pneumocytes of the alveolar epithelium. No type II pneumocytes were found. Cultured and fresh aspirates of bone marrow cells can express the type I pneumocyte markers T1α and aquaporin-5. Limited engraftment was found in the normal lung, but engraftment was facilitated after intra-tracheal bleomycin-induced lung injury. 1-2 million cells were transplanted into the tail vein 5 days after bleomycin treatment. At 1, 2.5, 5, 14, or 30 days after marrow injection, mice were sacrificed and analyzed. LacZ-labeled cells showed morphological characteristics of type I pneumocytes in that they presented as flattened cells lining the lung alveoli with ovoid nuclei that bulged slightly into the alveolar lumen and were adjacent to type II pneumocytes. Kotton et al. [8] listed three differences as possible sources of the discrepancy between their results and those of Krause et al. (1): use of recipients with intact bone marrow, induction of injury with bleomycin, and selective expansion of plastic-adherent cells.

Ortiz et al. [24] studied the donation of murine bone marrow MSCs from male mice donors intravenously via the tail vein into female mice treated with bleomycin. Male DNA accounted for 2.21 x 10-5 percent of the total lung DNA in control mice but increased 23-fold ($P<0.05$) in animals exposed to bleomycin before MSC transplantation. The engraftment of MSCs to form epithelial type II lung cells increased 62-fold compared to untreated controls. The intra-tracheal exposure of mice to bleomycin but not saline resulted in subpleural areas of inflammation that encompassed 55-60% of the lung parenchyma, producing consolidation with loss of normal alveolar architecture, also involving the bronchi and vasculature. Administration of MSCs significantly reduced this inflammation. Delaying the administration of MSCs did not alter the engraftment, but eliminated the anti-inflammatory effects. This early paper indicating a role in MSCs in altering immune function has become the subject of considerable interest (see discussion below). Bleomycin treatment caused marked alteration in lung architecture, with increased cellularity and fibrosis. BMDMSC incorporated, forming fibroblasts, type I alveolar cells, type II alveolar cells, and myofibroblasts. After bleomycin treatment, about 29% of the lung cells were derived from donors. In bleomycin-treated animals with intact marrow, less than 5% of the cells were derived from donors.

Hashimoto et al. [25] studied the origin of fibroblasts in pulmonary fibrosis. Adult mice were durably engrafted bone marrow isolated from transgenic mice expressing enhanced GFP production. Induction of pulmonary fibrosis in these chimera mice by endotracheal bleomycin injection caused large numbers of GFP-positive cells to appear in active fibrotic lesions, while only a few GFP-positive cells appeared in control lungs, demonstrating that collagen-producing lung fibroblasts in pulmonary fibrosis can be derived from bone marrow progenitor cells.

Rojas et al. [26] transplanted bone marrow-derived mesenchymal stem cells from donor male mice intravenously via the tail vein to mice whose lungs had been injured with bleomycin injected into the tracheal lumen. Myelosuppression increased susceptibility to bleomycin protection. Protection was associated with differentiation of the BMDMSC into specific and distinctive lung cell phenotypes. In vitro, cells from injured (but not normal mouse lung) produced soluble factors that caused BMDMSC to proliferate and migrate towards the injured lung. BMDMSC localize to the injured lung and assume lung cell phenotypes, but protection from injury and fibrosis also involves suppression of inflammation and triggering production of reparative growth factors. Bleomycin treatment caused marked alteration in lung architecture, with increased cellularity and fibrosis. BMDMSC incorporated, forming fibroblasts, type I alveolar epithelial cells, type II alveolar epithelial cells, and myofibroblast cells. In animals receiving bleomycin after myelosuppression, about 29% of the lung cells were derived from donors. In bleomycin-treated animals with intact bone marrow, less than 5% of the cells were derived from donors. When stem cells were co-cultured with cells from lungs obtained 14 days after bleomycin treatment, there was a marked proliferation of the stem cells, which migrated toward the lung cell suspensions. Neither of these effects were noted with cell suspensions from the lungs of animals that had not received bleomycin. Rojas et al. (25) hypothesized that the generation of humoral mediators by the injured lung could cause local proliferation of stem cells mobilized from bone (or delivered as a stem cell transplant) to expand the production of chemotactic substances and may also produce homing of stem cells to areas of tissue injury.

Ortiz et al. [27] followed their previous study (24) that showed that MSCs were efficacious in ameliorating lung injury only when administered at the time of bleomycin challenge, and not at later time points, suggesting that the therapeutic effect of MSCs was attributed to the production of soluble factors that modulate inflammation. Ortiz et al. [27] identified murine and human MSC subpopulations that secrete high levels of interleukin 1 receptor antagonist (ILIRN). They provided evidence that production of ILIRN by MSCs protects mice from bleomycin-induced lung injury by blocking the production and/or activity of TNF-alpha and IL-1-alpha, the predominantly pro-inflammatory cytokines in lung tissue. They suggested that this subpopulation of MSCs that express ILIRN provides a basis for developing MSC-based therapies to treat interstitial lung disease. Thus, this paper belongs to a second theme, MSCs as modulators of immune function (see discussion below).

Moodley et al. [28] studied the effects of injection of human mesenchymal stem cells derived from the umbilical cord (uMSCs) into the tail vein of mice subjected to a bleomycin-induced lung injury model. They found that the umbilical stem cells did not

differentiate into lung epithelial cells in vitro. uMSCs were found in the mouse lung at 14 days, but there was no evidence of their presence at 28 days following injection.

Other Models of Injury

Alkylating Agent-Mediated Lung Injury

Reese et al. [29] showed that cells in the bone marrow expressing the drug resistance gene MGMT can engraft in the lung and convert into cells expressing the type II pneumocyte surfactant protein C (SP-C). The number of these cells can be increased in response to lung injury produced by an alkylating agent. Increase the number of donor-derived hematopoietic cells that have characteristics of type II pneumocytes by overexpression of the drug resistance gene methylguanine DNA methyltransferase (MGMT). MGMT encodes O^6–alkylguanine DNA alkyl-transferase (AGT), a drug resistance protein for DNA damage induced by N,N'-bis(2chloroethyl)-N-nitrosourea (BCNU), and the mutant P140K MGMT confers resistance to BHCNU and the ACT inactivator O^6–benzylguanine (BG). Two models were used: one in which the donor cells had a strong selection advantage because the recipient lung lacked MGMT expression, and another in which drug resistance was conferred by gene transfer of P140K MGMT. In both models, BCNU treatment resulted in an increase in the total number of donor-derived cells in the lung. Donor-derived cells in the lung are rare and can be enriched with BCNU treatment. In the absence of BCNU injury, 16% of the cells present in the lung of MGMT-/- mice receiving GFP transgenic whole bone marrow were of donor origin at 3 months after transplantation. Donor cells included hematopoietic and parenchymal cells. Donor-derived SP-C+ cells were detected in two of the mice at frequencies of 0.10% and 0.09% respectively. The three other mice showed no evidence of SP-C+ cells. In untreated mice, 20.3% of cells harvested from the lung were donor-derived at 3 months after transplantation, and two of the six mice had donor-derived frequencies of 0.06% and 0.005%. After BG and BCNU treatment, 25% of cells from the lung were of donor origin. Two of the donor mice receiving this treatment exhibited donor-derived SP-C+ cells. Nine other mice exhibited no evidence of SP-C+ cells. In mice with evidence of donor-derived SP-C+ cells, the frequency increased after selection to 0.38% of total cells or 5.4% of all SP-C+ cells in one mouse and to 0.12% of total cells, or 1.96% of all SP-C+ cells. In one of these mice, the relative cell size and granular SP-C staining was consistent with a type II pneumocyte. These numbers suggest that the occasional bone marrow-derived cells may differentiate into cells with characteristics of pulmonary epithelial cells, and their numbers can be increased by treatment with BCNU. However, the lack of detection of these cells in all mice suggests that the capable progenitor is present in low frequencies, perhaps rare under the conditions of transplantation.

Asbestos-Induced Pulmonary Fibrosis

Spees et al. [30] found rare bone marrow-derived type II cells in the lung parenchyma of adult female rats that were lethally irradiated and rescued by bone marrow transplants from male transgenic rats expressing GFP. Three weeks later, rats were exposed to an asbestos aerosol. Type II alveolar cells were identified by surfactant protein C (SPC) immunocytochemical staining. The bone marrow-derived type II cells made up less than 0.5% of the total SPC-positive cells.

Busulfan/Total Body Irradiation Model

Bruscia et al. [16] studied the engraftment of donor-derived epithelial cells in multiple organs following bone marrow transplantation of 5-10 million cells in newborn mice. Animals were sacrificed at 80-100 days of life. Rare donor-derived epithelial cells were found in the gastrointestinal tract and lung but not liver of these animals. The mean of the bone marrow-derived epithelial cell frequencies for each experimental group was as follows: Controls exhibited 0.003% engraftment. A second group of mice was injected with 15 mg/kg busulfan on both days 17 and 18 of pregnancy, resulting in 0.02% engraftment in the lung. A third group was subjected to 15 mg busulfan on day 19 of pregnancy and these same pups were subjected to 400 cGy of irradiation during the first postnatal day, resulting in 0.009% engraftment. A fourth group of 1 day-old pups was subjected to 400 cGy irradiation alone. No engrafted cells were observed in this group. Bruscia et al. [16] concluded that bone marrow-derived cells can engraft as functional epithelial cells during the physiological growth and expansion of tissues in newborn mice and that these levels are similar to those observed during the tissue repair process in adult life.

Detergent-Induced Lung Damage

MacPherson et al. [31] subjected female mice to lethal irradiation (1050 rads) and rescued them by intravenous delivery into the tail vein of either 10,000 side population cells or 10 million bone marrow cells from male *ROSA26* mice. Side population (SP) cells are isolated by their ability to exclude the DNA binding dye Hoechst 33342. Side population cells are potent hematopoietic cells. Even a single SP cell is capable of reconstituting the hematopoietic system. (1000 fold less). Animals were housed for 3 months. The tracheas were damaged by instilling 10 microliters 2% polidocanol or polidocanol plus 1 mg/ml E. Coli lipopolysaccharide applied intra-nasally. All animals were harvested 7 days after the polidocanol instillation. Y chromosome FISH analysis was used to determine the number of Y chromosome positive cells in the tracheal epithelium. In the whole marrow-treated animals with no tracheal damage, no significant donor-cell contribution to the epithelial layer was seen. Only 0.14% of cells gave a Y chromosome-positive signal. The frequencies of engraftment of male cells in the tracheas of mice ranged from 1.0-1.6% with whole marrow and 0.6-1.5% with SP cells. The majority of these cells express cytokeratin, an epithelial marker. 15.9% of the cells with Y chromosomal damage were CD45 positive cytokeratin-negative cells which is consistent with an inflammatory phenotype.

Double Transgenic Mice with Doxycycline-Dependent Lung-Specific Fas Ligand Overexpression, and Treated with Doxycycline as a Model of Neonatal Lung Injury

De Paepe et al. [32] investigated the capacity of human cord blood-derived CD34-positive hematopoietic progenitor cells to regenerate injured alveolar epithelium in newborn mice. Double transgenic mice with doxycycline (Dox)-dependent lung-specific Fas ligand

overexpression were treated with Dox between embryonal day 15 and postnatal day 3. The dox-induced FasL upregulation produced by dox treatment resulted in dramatically increased apoptosis of alveolar type II cells and Clara cells, disrupted alveolar development, and increased postnatal lethality. Thus, FasL-induced alveolar epithelial apoptosis during postcanalicular lung remodeling was sufficient to disrupt alveolar development after birth. 100,000-500,000 CD34-positive cells were administered on postnatal day 5 by intranasal inoculation. Engraftment, respiratory epithelial differentiation, and cell fusion were studied at 8 weeks after inoculation. Engrafted cells were readily detected in all recipients and showed a higher incidence of surfactant immunoreactivity and proliferative activity in FasL-overexpressing animals compared with non-FasL-injured littermates. These authors concluded that CD34-positive cells derived from human cord blood are capable of long-term pulmonary engraftment, replication, clonal expansion, and reconstitution of injured respiratory epithelium. Cord blood-derived surfactant-positive epithelial cells appear to act as progenitors of the distal respiratory unit, analogous to resident type II cells.

Elastase-Induced Pulmonary Emphysema

Ishizawa et al. [33] studied mice with elastase-induced emphysema treated with agents known to reverse the anatomical and physiological signs of emphysema: all-trans-retinoic acid (ATRA), granulocyte-colony-stimulating factor (G-CSF), or both. Bone marrow-derived cell numbers were as follows: controls 0.8%, elastase plus vehicle 6.5%; elastase plus ATRA 17.8%; elastase plus G-CSF 20.87%; ATRA plus G-CSF 28.3% (interpolation from Figure 1 in Ishizawa et al. [33]).

Endotoxin

Beckett et al. [34] found that adult marrow-derived stem cells can localize to lung and acquire immunophenotypic characteristics of lung epithelial cells. Lung injury increases recruitment of the marrow-derived cells. We speculated that comparing patterns of lung engraftment following different lung injuries would provide insight into potential mechanisms by which marrow-derived cells were recruited to lung. To evaluate this, adult female C57Bl/6 mice irradiated and engrafted with marrow from adult male transgenic GFP mice were exposed to either intranasal inhalation of endotoxin (25 microgram/mouse) or 3 days of 25 ppm nitrogen dioxide and then compared 1 or 3 months later to transplanted but otherwise uninjured mice.

In all cases, the majority of marrow-derived cells recruited to lung were CD45-positive leukocytes. In lungs of transplanted but otherwise uninjured mice, small numbers of CD45-negative donor-derived cells in alveolar septae stained positively for pro-surfactant protein C. Rare donor-derived cells located in the airway epithelium stained positively with cytokeratin. Subsequent exposure of engrafted mice to niutrogen dioxide or endotoxin did not significantly increase the number or pattern of donor-derived CD45-negative cells found in recipient lungs. These results suggest that nitrogen dioxide or endotoxin lung injury does not result in significant engraftment of marrow-derived cells in lung.

Gupta et al. [35] showed that intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improved the survival and attenuated endotoxin-induced acute lung injury in mice. The beneficial effect of BDMSCs was independent of the ability of the cells to engraft into the lung. In vitro co-culture studies showed that the anti-inflammatory effect was paracrine and not dependent on cell contact.

Xu et al. [36] studied the prevention of the endotoxin-induced systemic response by bone marrow-derived mesenchymal stem cells in mice. Mice were subjected to an intraperitoneal injection of 1 mg/kg endotoxin followed by either 0.5 million BMDMSCs, 0.5 million fibroblasts, or normal saline. Lungs were harvested 6 hr,24 hr, 48 hr, and 14 days after endotoxin administration showed that BMDMSC administration prevented endotoxin-induced pulmonary inflammation, injury, and edema. Donor cells were detected in the lungs at day 1 after endotoxin administration, no donor cells were detected by day 14. BMDMSC administration suppressed the endotoxin-induced increase in circulating pro-inflammatory cytokines without decreasing the circulating levels of anti-inflammatory mediators.

Hyperoxia-Treated Newborn Mice

Fritzell et al. [37] studied the effects of adult bone marrow cells expressing green fluorescent protein administered intra-nasally to normoxic and hyperoxic (95%) newborn mice. Lungs were analyzed between day 2 and week 8. The volume of GFP-immunoreactive donor cells remained constant between post-transplantation weeks 1 and 8, and was similar in normoxic and hyperoxic-exposed recipients. Virtually all marrow-derived cells showed co-localization of GFP and the pan-macrophage marker F4/80 by double immunofluorescence studies. Re-exposure of marrow-treated animals to hyperoxia at postnatal day 66 resulted in significant expansion of the donor-derived macrophage population. The relationship between damage by hyperoxia translates compared with radiation or bleomycin-induced injury is a variable in comparing the results of this study with those discussed above, as is the neonatal administration. However, this study does bring up the issue of MSCs and regulation of activity of the immune system, which has become important in issues concerning MSC transplantation (see below).

Lipopolysaccharide-Induced Lung Injury

Yamada et al. [38] studied the effects of intranasal insufflation in mice of 20 micrograms in PBS of lipopolysaccharide (LPS), a component of Gram-negative cell walls. Rapid mobilization of BMPCs into the circulation was observed. These cells accumulate within the inflammatory site and differentiate to become endothelial and epithelial cells. Suppression of BMPCs by sub-lethal irradiation before intrapulmonary LPS leads to tissue disruption and emphysema-like changes. Reconstitution of the bone marrow prevents these changes, suggesting that BMPCs are important and required for lung repair after LPS-induced lung injury. Recipient mice were irradiated using 8Gy and 4 Gy, separated by 3 hours. Bone marrow was reconstituted by injecting 2 million fetal liver cells via the tail vein. Irradiated recipient mice were reconstituted

with bone marrow cells from green fluorescent protein (GFP) expressing transgenic mice to generate mice with GFP-expressing bone marrow cells. 3 weeks after reconstitution of bone marrow, LPS or phosphate buffered saline (PBS) was administered intranasally to recipient mice. Immunohistochemical analysis was performed to detect GFP expression. Thin, flat GFP-positive cells, morphologically consistent with alveolar epithelial cells or endothelial cells, appeared in the parenchyma of LPS-treated recipient lungs, but not in PBS-treated control lungs. These cells were not present before or at 4 hour after LPS. Only a few cells were present 24 hours after LPS treatment, but they were numerous at 1 week. CD45 staining was used to identify hematopoietic cells, or CD34 to identify endothelial cells. Flat GFP-positive cytokeratin positive CD45-negative cells were present in the alveolar walls of LPS-treated lungs, but not in control lungs, suggesting that BMPCs had differentiated into an alveolar epithelial phenotype. Flat GFP-positive CD34-positive CD45–negative cells were present, suggesting differentiation toward pulmonary capillary endothelial cells. Although CD45-positive cells were identified in the same lung sections, no cell stained simultaneously for both CD45 and cytokeratin, or for CD45 and CD34. BMPCs are required for normal repair of lungs damaged by inflammation induced by LPS.

Zhang et al. [39] utilized a surfactant-associated protein A suicide gene system, which induces apoptosis in alveolar epithelial type II cells, vacating the alveolar epithelial type II stem cell niche. This led to increased homing of MSCs to the lung in chronic obstructive pulmonary disease (COPD) rats. To establish a model of COPD, rats were given 0.2 ml of 1 mg/ml lipopolysaccharide by tracheal instillation on days 1 and 4, and passive cigarette smoking. Y chromosomes were detected by fluorescent antibody in situ hybridization (FISH). The increase in MSC homing is associated with collagen fiber deposition. Zhang et al. [39] hypothesized that it is not the MSCs themselves (whether endogenous or exogenous) but the perturbation of diseased AT II cell niches that alter the potential migration or differentiation of MSCs. Thus, hypoxia promotes fibrogenesis.

Lysosomal Acid Lipase Deficiency

Yan et al. [40] studied alveolar type II cells and Clara cells. About 10-18% of alveolar type II epithelial cells exhibited positive lacZ gene expression after 8 weeks of BMSC injection in recipient ¬-/- mice. Wild type mice exhibited no expression after the same treatment. Pulmonary inflammation triggered by lysosomal acid lipase (LAL) deficiency can trigger BMSC residing in lal -/- bone marrow, migrating into the lung and converting into residential AT II epithelial cells. LAL knockout mice. Once being recruited into the lung, adherent BMSCs showed the ability to convert into AT II epithelial cells. Since this conversion did not occur in the wild-type lung, pulmonary inflammation triggered by LAL deficiency is an important factor for the recruitment of adherent BMSCs. It seems that neutrophil influx can serve as an indicator for adherent BMSC release in disease conditions. lacZ used as a reporter gene. lacZ encodes beta-galactosidase. The hSP-B 1.5-kb lacZ reporter gene was used as a specific marker for identifying AT II epithelial cells in the lung. The absence of lysosomal acid lipase in the mouse (LAL-knockout mouse) blocks cholesteryl ester and triglyceride metabolic pathways, resulting in massive release of inflammatory cells (neutrophils and monocytes) from the bone marrow into the lung. This inflammatory event evokes severe changes in the lung that resemble the effects of smoking, including hypercellularity and emphysema. Alveolar type II epithelial cells (AT II cells) are the major sites for the initiation of inflammatory changes seen in LAL deficiency.

Napthalene-Induced Airway Injury

Loi et al. [18] studied whether adult murine bone marrow-derived cells containing normal cystic fibrosis transmembrane conductance regulator protein (CFTR) could repopulate pulmonary epithelium of CFTR-knockout mice. To increase marrow recruitment naphthalene was used to induce airway epithelial injury in recipient mice. Determination of CFTR-mRNA was performed by reverse transcription-polymerase chain reaction (RT-PCR). Adult transgenic male mice expressing green fluorescent protein (GFP) were used as experimental donors. One million plastic-adherent bone marrow stromal cells were administered via tail vein. For transplantation of total marrow cells, adult female CFTR-knockout mice underwent total body irradiation (800 rads) followed by tail vein administration of 20 million CD3-depleted total bone marrow cells. Napthalene was administered via intraperitoneal injection to naive adult female CFTR-mice three days b before administration of cultured stromal cells. Another group of chimeric CFTR-female knockout mice received the same naphthalene treatment one month after transplantation of bone marrow cells. Plastic-adherent bone marrow stromal (MSC) cells were administered via tail vein injection to adult female CFTR-knockout mice with intact bone marrow. Mice were assessed one week, one month, and three months after transplantation. At each time point, rare donor-derived cells were observed in recipient mouse lungs. The majority of the cells was CD45-negative, located in the alveolar walls, and demonstrated morphological characteristics of alveolar epithelial and/or endothelial cells. Rare donor-derived cells expressing Clara cell secretory protein were detectable in airways of the recipient lung at all-time points after administration of bone marrow stromal cells. Non-injured animals demonstrated 0.33%, naphthalene after one week 1.0%, uninjured one month (0.5%), naphthalene 1 month1.5%, uninjured 3 months 2%, and naphthalene 3 month 5%. Airway remodeling after naphthalene injury approximately doubled the number of chimeric airway epithelial cells found at each time point, although the increase was not statistically significant. Clustering of Y-chromosome-positive, CD45 negative CCSP-positive cells were observed three months after naphthalene administration. 0.025 + 0.1% of the CCSP-positive cells were of donor origin. Donor-derived cells were assayed by fluorescence in situ hybridization (FISH) for Y chromosome-positive cells followed by immunohistochemical characterization of the epithelium and leucocytes using antibodies to Clara cell secretory protein (CCSP), pro-surfactant protein C (pro-SPC), cytokeratin, and CD45.

Serikov et al. [41] transplanted sex-mismatched green fluorescent stem protein (GFP)–tagged bone marrow derived cultured plastic-

adherent mesenchymal stem cells into sublethally irradiated (5 Gy) recipient mice. After one month of recovery, experimental animals were subjected to 250 mg/kg napthalene IP. Animals were killed 2-30 days later. 1 million MSCs were administered via the jugular vein. No green fluorescent protein (GFP)-positive cells were observed in the airway epithelium of non-injured chimeric animals transplanted with MSC. After naphthalene injury, patches of GFP-positive cells were present in the lung parenchyma and epithelium of conducting airways at 2-6 days after naphthalene. GFP-positive cells in the epithelium were positive for pancytokeratin, but not Clara cell secretory protein (CCSP). GFP-positive cells formed clear isolated patches of the bronchial epithelium, consistent with clonal formation, as they were also positive for proliferating cell nuclear antigen (PCNA), a marker for proliferating cells. At day 30, only very rare GFP-positive cell patches were present in the epithelium. Serikov et al. [41] postulated that after acute naphthalene-induced injury, BMSC participate in repair of pulmonary epithelium in a time-dependent manner. After initial development of patches in the epithelium, consistent with clonal formation, these cells nearly disappear from the epithelial lining at the time of complete recovery from injury.

Wong et al. [42] generated transgenic mice expressing green fluorescent protein (GFP) derived by the epithelial-specific cytokeratin-18 promoter. 200 mg/kg naphthalene was administered by intraperitoneal injection to induce airway injury, since this agent is specific for damage to Clara cells. BMC from these mice were injected trans-tracheally into wild-type recipient mice after naphthalene-induced airway injury. BMC retention in the lung was observed for at least 120 days following delivery with increasing GFP transgene expression over time. Wong et al. hoped that the use of this "conditioning regimen" to deplete the progenitor Clara cells, thereby creating an airway-specific niche for cell incorporation. Mice were killed at 1, 4, 7, 14, 30, 60 or 120 days after BMC injection. BMC were labeled with CellTracker Orange. These labeled BMC were easily detected in the distal airways and alveoli of naphthalene-treated lungs. Two days after delivery 2-5% of the BMC were positive and also colocalized CCSP. At 14 days after cell delivery (16 days after naphthalene-induced lung injury), donor-derived cells formed 3.35% of the total number of CCSP-positive cells. Wong et al. [40] estimated that the total contribution of the BMC cells to the airway epithelium after trans-tracheal delivery would be at most 1% after complete airway epithelial regeneration.

Giangreco et al. [43] demonstrated that airway stem cells do not contribute significantly to lung homeostasis, but rather their activation is contingent upon epithelial progenitor cell depletion following severe lung injury. The bronchiolar stem cell compartments of the conducting airways of green fluorescent protein chimeric mice were microdissected, immunostained, and studied using a lung whole-mount imaging method. They found that abundant progenitor cells regulate epithelial maintenance during normal airway homeostasis. Stem cell activation is not required. For injury studies, animals were divided into slightly injured (<10% loss of body weight 3 days after napthalene administration) and severely injured (>10% loss of body weight 3 days after naphthalene administration). The lungs of all severely injured animals recovered for 30 days exhibited a very unique pattern of very large, GFP-positive cell patches localized to airway branchpoints and terminal bronchioles, demonstrating the involvement of airway stem cells in the repair of injury.

Wong et al. [44] identified by flow cytometry a subpopulation of human and murine adherent bone marrow derived stem cells that can contribute to repair of the pulmonary epithelium. These cells express Clara cell secretory protein (CCSP). When cultured at the air-liquid interface in ex vivo cultures, CCSP-positive cells expressed markers for type I and type II alveolar cells as well as markers for basal cells and active epithelial sodium channels. When delivered intra-tracheally or intravenously, these cells preferentially homed to naphthalene-damaged airways. Intra-tracheal instillation was more efficient than intravenous administration. Napthalene-induced lung damage transiently increased CCSP expression in bone marrow and peripheral circulation. Lethally-irradiated CCSP-null mice that received tagged wild-type bone marrow contained donor-derived epithelium in both normal and naphthalene-damaged airways. These cells were isolated, proliferated in culture and increased in bone marrow and peripheral blood in response to airway injury. The majority of these CCSP-positive cells expressed CD45 and mesenchymal markers CD73, CD90, and CD105. They differentiated into multiple epithelial cell lineages, including both airway and alveolar lineages following air-liquid interface culture. The population of bone marrow cells that expressed CCSP (1.9%) expanded in culture up to 25.8% after 7 days. CCSP-positive cells were smaller, more rounded than CCSP-negative cells which were larger with more cytoplasmic extensions. CCSP-positive cells expressed the pan-hematopoietic marker CD45, and the progenitor marker CD34. Both CCSP-positive and CD-negative cells expressed the MSC markers CD73, CD90, and CD105. CCSP-positive cells did not express the MSC markers CD106, type I collagen, or type IV collagen. Some CCSP-negative cells expressed CD106, type IV collagen and type I collagen. CCSP-negative cells expressed all the mesenchymal stromal cell genes but no hematopoietic genes. Morphologically, CCSP+ cells were small (about 5-10 micrometers) rounded cells, with no or few cytoplasmic extensions. CCSP-negative cells were greater than 10 micrometers in size. They varied from very large cells with considerable cytoplasm to medium-sized cells with large cytoplasmic extensions.

Pseudomonas aeroginosa

Rejman et al. [45] investigated the effect of epithelial damage caused by *Pseudomonas aeruginosa* (a bacterium widely occurring in cystic fibrosis), on the engraftment of bone marrow cells in airway epithelium. Intravenous (none) or intra-tracheal (few) administration of unfractionated green fluorescent protein (GFP) positive bone marrow cells in mice infected with *Pseudomonas aeruginosa* resulted in none or very few GFP+ cells in the lungs of recipient mice. Significant numbers of GFP-positive cells were observed only when GFP-positive cells were purified to obtain a cell suspension enriched in progenitor cells and injected intra-

tracheally (0.5 to 1 million cells). Localization of the donor cells at the level of airway epithelium was confirmed by FISH analysis for the Y chromosome. All donor-derived Y chromosome-positive cells were found to express cytokeratin. The fractions of GFP+ cells expressing cytokeratin were 0.60% (100,000 colony-forming units (CFU) bacterial cells administered) or 1.12% (one million CFUs of bacterial inoculums. Thus, the result was proportional to the number of CFUs administered. Thus, Rejman et al. [45] demonstrated that the tissue damage inflicted by bacteria such as *Pseudomonas aeruginosa* can facilitate the airway engraftment of heterologous bone marrow-derived stem cells and their epithelial transformation. CD45 expression was limited to infiltrating leucocytes.

Human Studies: Sex Mix-Matched Human Lung Transplants (Female Recipients, Male Donors)

Kleeberger and colleagues [46] examined chimerism within the epithelial structures of the lung using Y-chromosome in situ hybridization. Utilizing tissue biopsies from explanted gender-mismatched human lung allografts (n=7), they found integration of recipient-derived cells in the bronchial epithelium (6 to 26%), type II pneumocytes (9 to 20%) and seromucous glands (9 to 24%). While not statistically significant, the degree of chimerism also appeared to be related to the extent of injury, wherein the highest proportion of recipient cells was found in lungs exhibiting pronounced epithelial metaplasia associated with bronchitis. The researchers also found no evidence that the donor-derived cells were bone marrow derived in the lung biopsies of three bone marrow transplant recipients.

Suratt et al. [47] studied lung specimens from three female allogeneic cord blood transplant recipients who received stem cells from male donors. Significant rates of epithelial (2.5-8%) and endothelial (37.5-42.3%) chimerism were detected.

Mattsson et al. [48] demonstrated engraftment after allogeneic hematopoietic stem-cell transplantation in lung tissue specimens of four female patients, in which two of the donors were male. Complete chimerism in all cell lineages was found in all patients. Two and 6% Y-chromosome-positive epithelial lung cells were found in the two positive controls, as well as detected engraftment of type II pneumocytes.

Spencer et al. [49] studied trans-bronchial lung biopsies from male patients with cystic fibrosis who received heart-lung transplants from female donors. Co-localization of Y-chromosomes and cytokeratin staining was found in bronchial epithelial cells (1.47% average, range 0-9.6%), and in alveolar epithelial cells (3.6% average, range 2.3-3.5%).

Albera et al. [50] examined lung specimens from eight male patients who received female donor lungs and three female patients who had received male bone marrow transplants. They found Y-chromosomes in the alveolar epithelium, some of which had differentiated into type II pneumocytes. Y-chromosomes were also found in macrophages and endothelial cells, which are of mesenchymal lineage.

Zander et al. [51] studied the effects of lung transplants from male donors to female recipients in terms of the number of type II pneumocytes. Of the four female patients, only one patient exhibited Y-chromosome-containing-type II pneumocytes. When the effects of incomplete nuclear sampling were adjusted, this represented 1.75% of all type II pneumocytes.

Human Studies: Cell Culture

Spees et al. [52] tested the hypothesis that human mesenchymal stem cells (hMSCs) might respond to tissue injury by differentiating to form new cells of the injured type. Confluent cultures of small airway epithelial cells (SAECs) were heat-shocked at 47°C for 30 minutes to induce cell damage and death. They were then co-cultured with human mesenchymal stem cells (hMSCs). After heat shock, the majority of the SAECs remained adherent but many cells lost cell-cell contact as they underwent cytoplasmic retraction, opening up holes in the monolayer. Green fluorescent protein-positive (GFP+) hMSCs from an isolated clone were added 1-2 hours after the heat-shocked SAEC cultures had cooled to 37°C. Within 12 hours, about one percent of the adherent hMSCs had begun to lose their characteristic fibroblast morphology and had become flattened and translucent with an epithelial shape that manifested as a broad, flattened cytoplasm and an elevated perinuclear region. After 24 hours, many of the GFP+ hMSCs were indistinguishable from SAECs by phase-contrast microscopy. By 48-96 hours, a continuous monolayer was reassembled in the cultures to which the hMSCs were added. By contrast, when hMSCs were added to cultures of non-heat-shocked SAECS, the hMSCs showed little evidence of differentiation. When heat-shocked SAECs were cultured alone, they did not consistently regain confluency. The morphologically differentiated GFP-positive hMSCs were positive for several epithelial-specific markers such as keratins 17,18, and 19, as well as CC26 (a marker for Clara, serous and goblet cells in the lung). Immunocytochemistry for E-cadherin and β-catenin demonstrated that differentiated GFP-positive hMSCs formed adherens junctions with SAECs. The undifferentiated GFP-positive hMSCs in the same co-culture were negative for keratins and CC26. Also the undifferentiated GFP-positive hMSCs did not stain for E-cadherin, stained very lightly for β-catenin, and did not form pseudostratified epithelioid associations characteristic of SAECs. These studies provide evidence that cultured hMSCs can be a source of cells to repair damaged epithelium.

Wang et al. [53] utilized cells from human bone marrow stroma expressing green fluorescent protein (GFP). When human MSCs expressing GFP were co-cultured with human airway epithelial cells, the spindle-like MSCs changed to a columnar epithelial-like shape. Co-localization of GFP and cytokeratin staining demonstrated that some of the MSCs had changed to airway epithelial cells.

Discussion

Emphasis on Improved Technical Approaches to the Identification of Putative Alveolar Cells that are Derived from Exogenous Stem

Cells Following Injury.

Initial reports found that administration of bone marrow-derived stem cells led to engraftment into the lung of recipient mice [2,8]. Negative reports appeared as well (5,10). After the initial report by Kotton et al. [8] demonstrating incorporation of bone marrow-derived stem cells into the lung as type I alveolar pneumocytes, these authors found that technical difficulties had skewed the results. They concluded [14] that the degree of incorporation was not significantly more than controls once autofluorescence, dead cells, and contaminating blood cells were accounted for. A number of technical challenges in the detection of bone marrow-derived epithelial cells have been identified: (1) proving that a cell donor derived by the use of markers such as the Y-chromosome or a donor-specific gene (either endogenous or a transgene), (2) proving that a cell is truly epithelial by the expression of cell-specific markers, and (3) ruling out overlay of cells [12,23]. Technical criteria for identifying stem cells that have undergone engraftment have been discussed by Kassmer and Krause [20].

The number of pulmonary alveolar cells that are found in the lung following transplantation has decreased somewhat from the earliest reports. These results may perhaps be related to the emphasis on improved technical approaches. However, the majority of the studies reported in this paper demonstrate a low but fairly consistent engraftment of exogenous stem cells into the lung following injury. Objections have appeared, however, that even if rare engraftment of stem cells occurs, the levels are too low to be of meaningful physiological or clinical significance [14,54,55].

Lung Injury is Required for Engraftment of Stem Cells to Form Pulmonary Alveolar Cells

Pulmonary damage is required for engraftment of bone marrow-derived stem cells into the lung to form epithelial cells [7,17,23,41]. There is a strong correlation between lung damage and the engraftment of bone marrow-derived stem cells to form pulmonary alveolar cells [12,23].

There is a threshold of lung injury that is required for the appearance of marrow-derived epithelial cells in the lung [17]. With radiation doses less than 800 centigray, no marrow-derived type II pneumocytes were identified. Doses greater than 1000 centigray led to the appearance of marrow-derived type II pneumocytes in at least 50% of mice.

Krause et al. [2] postulated that the stem cells are “summoned” to sites of injury by factors secreted from the damaged organ. The assumption is that some cells in the dying tissue of the lung act as signals to promote entry of bone marrow-derived stem cells to the damaged lung where they incorporate into the lung. Presumably they can reproduce and serve as a pool whereby new progenitor cells can repair the damaged tissues.

Role of Species

The engraftment of exogenous stem cells to form pulmonary alveolar epithelial cells has been demonstrated in various species such as mice, rats, and humans. Thus, the incorporation of bone marrow-derived stem cells is not dependent on species. The demonstration of incorporation into humans demonstrates that this mechanism has the potential to be useful for clinical purposes.

Sources of Controversy

Sources of controversy are as follows: (1) whether the findings themselves are actually detection artifacts and thus do not represent true bone marrow-derived epithelial cells, (2) that the frequency of bone marrow-derived epithelial cells is too low to be physiologically relevant, and (3) that the primary mechanism responsible for the appearance of bone marrow-derived epithelial cells is cell fusion [12,23]. However, Kassmer et al. [22] transplanted very small embryonic-like cells (VSELs) from donor mice expressing H2B-GFP under a type 2 pneumocyte-specific promoter, thereby demonstrating that engraftment occurs by differentiation and not by fusion.

VSELs

Ratajczak et al. [56] postulated that very small embryonic-like stem cells (VSELs) could be a link between the early stages of development and adult stem cell compartments, where they reside in a quiescent state. The epigenetic mechanism identified that changes expression of certain genes involved in insulin/insulin-like growth factor signaling in VSELs keeps these cells quiescent in adult tissue.

VSELs: Which Type of Stem Cell is the Most Effective in Producing Engraftment?

It has been hypothesized that VSELs are developmentally related to primordial germ cells (PGCs), and that a subpopulation of EpiSCs gives rise to VSELs during development [22].

Kassmer et al. [22] compared the ability of Oct4-positive VSEL stem cells and of bone-marrow-derived (BMD) stem cells to form epithelial cells in the lung. Transplantation with 900-1500 VSELs resulted in formation of 4% of all type 2 pneumocytes. Transplantation with 100,000 non-VSEL stem cells (from the non-hematopoietic bone marrow fraction), resulted in formation of only 0.36% of type 2 pneumocytes in recipient mice. These transplantations consistently gave rise to surfactant-positive epithelial cells in surfactant C-knockout mice.

Kassmer et al. [21] showed that non-hematopoietic (lineage negative) bone marrow cells are the primary source of bone marrow-derived pulmonary epithelial cells by showing that they consistently gave rise to surfactant protein C-positive pulmonary epithelial cells in SPC knockout mice (SPC-KO) mice, whereas hematopoietic bone marrow cells did not. Kassmer et al. [22] used this model to identify the subpopulation of non-hematopoietic bone marrow cells that are capable of giving rise to pulmonary epithelial cells. They found that in the mouse, very small embryonic-like cells (VSELs) are small (4-6 microns in size). They are lineage-negative, CD45-negative stem cells in the bone marrow that express Oct4 and Nanog and give rise to cells belonging all three germ layer lineages in vitro [56,57].

Thus the VSELs are the cell type that is primarily involved in the formation of type II pulmonary alveolar cells when exogenous bone marrow cells are transplanted into damaged mice. Since the type II pulmonary alveolar cells are of the endodermal lineage, VSELs have broad differentiation potentials [22].

Possible Multiple Mechanisms of Action of Exogenous MSCs or VSELs

It is possible that exogenous MSCs or VSELs can engraft into the lung, where they act through multiple mechanisms. Four possibilities are considered by Caplan [58] for the actions of trophic mediators secreted by MSCs: (1) inhibition of ischemia-caused apoptosis; (2) inhibition of scar formation; (3) stimulation of angiogenesis and vessel stability; and (4) stimulation of mitosis of tissue-intrinsic progenitor cells. These progenitor cells would then produce differentiated pulmonary cells thereby regenerating pulmonary tissues. The alveolar epithelial cell type 2 is the best candidate for the progenitor cell of the alveolar compartment [59]. Hypothesis 4 has the advantage of explaining how a relatively small number of cells from exogenous sources incorporated into the lung could exert a disproportionate effect on the regeneration of the lung without having to end up directly differentiating into progenitor cells. The progenitor cells (which would lack the genetic label of the MSCs) could then differentiate into target cells as needed to regenerate the damaged tissues. Thus, the exogenous stem cells could act to augment the progenitor cell pool without necessarily giving rise through cell division to progenitor cells through cell division and differentiation. Thus, some of the labeled cells might form progenitor cells directly, in which case the progenitor cells would exhibit the green fluorescent protein in their genome. Others could stimulate progenitor cell division without involving cell division and differentiation, in which case they would not exhibit the green fluorescent protein in their genome. MSCs have also been implicated in the prevention of pulmonary damage by modulating the immune system [35,59-61].

Maintenance Cells as Paracrine Signaling Cells

Another aspect of mesenchymal stem cell use for therapy for acute lung injury involves their role as paracrine cells that secrete factors that can regulate endothelial and epithelial permeability, decrease inflammation, enhance tissue repair, and inhibit bacterial growth [61,62]. Thus, it may well be that exogenous stem cells act through multiple mechanisms. Alternatively, one particular cell might act through one mechanism, and a different stem cell through a different mechanism.

Caplan [58] proposed that engraftment and differentiation is a "1990s thinking/paradigm". The objection was originally raised that administration of bone marrow-derived stem cells did not result in engraftment [6,12,14]. However, more recent publications propose that although the phenomenon exists, it is too insignificant to be useful clinically [24,63-65]. However, such a judgment may be premature. First, the information about which cell type is more useful is very limited. VSELS appear to be more efficacious, but it is uncertain whether an even more primitive stem cell (perhaps a totipotent stem cell) could be more effective. For example, mesodermal stem cells should form cells of the mesodermal lineage such a bone, muscle, and tendon. Some studies have provided evidence consistent with this hypothesis. However, over time it gradually has become more likely that a subpopulation of bone marrow-derived stem cells was more effective. The only study that used a clonal population was the original work of Krause, in which the bone marrow was re-established following transplantation of a single cell. The absence of use of clonal populations of stem cells limits the conclusions that can be drawn from such studies. Second, the conditions required to optimize engraft and differentiation have not been rigorously studied. A time course and dose-response curve could do much to establish the optimum conditions. Moreover, it is possible that multiple doses are required to achieve optimum results. One would suspect that there may be a time window in which whatever signal molecules that stimulate homing of the stem cells to the injured tissues are probably released fairly early after damage. It would be helpful to know the time course of the signaling response to damage to the lung. Future development of molecules that can stimulate apoptosis could be a major development if the signal molecules are released during the apoptotic process.

Conclusion

We hypothesize that exogenous healing cells can undergo the differentiation cascade to form progenitor cells that from differentiated pulmonary alveolar cells directly, but that maintenance cells can also act through other mechanisms such as stimulation of mitosis of progenitor cells and perhaps also by paracrine actions as well. This hypothesis has the advantage of explaining how a relatively small number of cells from exogenous sources could exert a disproportionate effect on the regeneration of the lung without necessarily directly differentiating into progenitor and pulmonary alveolar cells. This hypothesis thus obviates the objection that the number of stem cells engrafted to form pulmonary alveolar cells is too small to be physiologically important.

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