Bone Marrow–derived Cells and Stem Cells in Lung Repair

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Although it has been many years since publication of the first peer-reviewed studies showing that bone marrow (BM)–derived cells can become mature-appearing epithelial cells, we still know very little regarding the mechanisms, kinetics, cells, and potential clinical utility or pathology associated with this phenomenon. The initial discovery of BM-derived epithelial cells (BMDE) in the liver was published by Petersen and colleagues (Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP. Bone marrow as a potential source of hepatic oval cells. *Science* 1999;284:1168–1170). Since that time, BMDE were identified in the skin, eye, GI tract, kidney, and the lung. Surprisingly, once several laboratories started to examine the effects of BM cells after tissue injury, BM-derived cells of different types were found to decrease tissue injury and enhance tissue repair, often without engraftment of marrow-derived epithelial cells. Thus, the potentially beneficial effects of BM-derived cells in some tissue microenvironments may be unrelated to differentiation into nonhematopoietic cell types. Here, I focus on recent findings from my laboratory as well as several other laboratories on the effects of BM cells on lung damage, and BMDE in the lung, including tracheal epithelial cells, bronchiolar epithelial cells, and type II pneumocytes in the alveoli. Potential mechanisms underlying the appearance of marrow-derived epithelial cells, and the role of tissue damage are discussed.

Keywords: stem cells; plasticity; tissue repair

TISSUE DAMAGE IS NECESSARY FOR APPEARANCE OF BONE MARROW–DERIVED EPITHELIAL CELLS IN THE LUNG

In many of the studies using bone marrow (BM) transplantation to study the BM to epithelial transition, BM-derived epithelial cells (BMDE) developed after some form of tissue injury. In the early studies, BM cells were transplanted into female mice that had undergone marrow-lethal whole body irradiation as a preparative regimen to promote BM engraftment, and, at least for engraftment of type II pneumocytes, the kinetics of appearance of BMDE coincided with the initial severe pneumonitis caused by high doses of radiation. The radiation level to which the mice were exposed (1,200 cGy) is known to cause severe lung injury characterized by breakdown of capillaries within alveolar septa and extravasation of erythrocytes into the alveolar spaces at

about Day 3, worsening until Day 5, and then restoring alveolar septal integrity by about Day 7 (1–3). BMDE were first detectable in the damaged alveolar tissue 5 to 7 days after lethal irradiation, and clusters of BMDE were detectable in alveoli by 2 months and remained relatively constant thereafter (4). BMDE can also be obtained with sublethal (< 1,000 cGy) irradiation; however, the dose needs to be above a threshold that causes lung damage (5). We transplanted BM into recipients that had received a preparative regimen of 400, 600, or 1,000 cGy, and within 1 month, all of the mice had greater than 85% hematopoietic engraftment in the BM and peripheral blood. We found a strong correlation between lung damage and the presence of BMDE. In mice that were transplanted after receiving 400 or 600 cGy, hematopoietic engraftment was high (> 85%), but there was no lung injury, and no marrow-derived epithelial cells. In contrast, in the mice that received 1,000 cGy irradiation there was significant lung injury, and BM-derived cells did engraft as lung epithelial cells in addition to engrafting the hematopoietic system. These data indicate a critical role for lung injury in the phenotypic change from BM cells to lung epithelial cells.

DETERMINING WHICH BM SUBPOPULATIONS ARE ABLE TO ENGRAFT AS EPITHELIAL CELLS

It is not clear which BM subpopulation(s) is (are) capable of engraftment as epithelial cells. The BM contains at least two different populations of stem cells, each of which is capable of both self-renewal and differentiation down multiple lineages. Hematopoietic stem cells (HSC) can differentiate into all blood cell types. In mice and humans, multiple approaches have been used to isolate and characterize HSC. Many isolation protocols start with a lineage depletion step, in which all cells that have already committed to a specific hematopoietic lineage are removed using a cocktail of different antibodies against surface antigens that are expressed following commitment (e.g., Glycophorin A expression on cells committed to the erythroid lineage). Additional enrichment steps have included (1) exclusion of rhodamine and Hoechst dyes (6), (2) expression of Sca1 and Kit antigens (7), and (3) the ability of HSC to home rapidly to the BM after intravenous infusion (8).

In addition to HSC, BM contains marrow stromal cells, also known as mesenchymal stem cells (the acronym MSC can signify either nomenclature). A major limitation in this new field of BM to epithelial differentiation is the lack of characterization of cell population studies used in each study. No specific constellation of surface markers has been agreed upon for these cells. Based on commonalities among several different manuscripts (9–12), a consensus statement has been published suggesting that the constellation of surface antigens on MSC includes CD13, CD44, CD73, CD90, CD105, CD106, and CD124, and that these cells should not express CD45 (13). Although the term “MSC” is used by many different laboratories, the cells vary from one lab to

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another. Cells referred to as MSC share a minimum of three primary characteristics: they grow as adherent cells in tissue culture plates; have a finite lifespan of approximately 30 to 50 cell doublings; and they can differentiate, under appropriate specific *in vitro* conditions, into osteoblasts, chondroblasts, and adipocytes. Variations in approaches used to grow MSC have led to variable findings regarding the differentiation potential of these cells in different laboratories. Importantly, some published reports report only that they used adherent BM cells, and do not provide adequate characterization of the cells transplanted for one to know whether these cells were adherent hematopoietic cells (e.g., CD45+ macrophages), endothelial cells, fibroblasts, or a mixture of many cell types. These significant differences in study design and cell characterization likely underlie many of the apparent discrepancies in the literature. For example, some investigators report that MSC can differentiate *in vitro* into neuronal type cells, while others cannot obtain this phenotype (14). Several different laboratories have reported data suggesting that MSC or adherent cells grown from the BM can differentiate into multiple types of lung epithelial cells (Table 1) (15–22). Others have reported not detecting BMDE in the lung (23, 24).

BM subpopulations that have been enriched using different approaches have been tested for their ability to engraft as lung epithelial cells *in vivo*. When very small ($< 5 \mu\text{m}$) BM cells are selected by elutriation (E), lineage depletion (L), and their ability to home (H) to the BM of a lethally irradiated host, the resulting population (called ELH) is greatly enriched for HSC (8). Like the lineage-negative, Sca+, Kit+ (LSK) population, a single ELH cell can provide long-term hematopoietic reconstitution in about 20% of lethally irradiated recipients (7, 25). We have shown that a single BM-derived cell can differentiate into both hematopoietic cells and lung epithelial cells in mice (25). We transplanted

single male-derived ELH BM cells into lethally irradiated female mice. (Short-term hematopoietic reconstituting cells from female donors were co-transplanted with this single cell to allow the lethally irradiated recipients to survive.) In the mice that survived and achieved hematopoietic reconstitution from the single ELH cells, there were Y chromosome-positive (male-derived) cytokeratin-positive epithelial cells in the lung, thereby demonstrating that a single marrow-derived cell can engraft not only as blood but also as mature epithelial cells throughout the body. In an independent study using GFP to mark donor-derived BM cells, Wagers and coworkers (24) reported that when a single LSK cell engrafts the entire hematopoietic system, there are rare BM-derived hepatocytes in the liver, but no BM-derived epithelial cells were detected in the lung (24). There were several technical differences between the studies performed with ELH and LSK cells. Whether LSK and ELH have the same ability to become BMDE has not yet been directly tested. That is, these populations have not yet been tested side-by-side in the same experiment using identical mouse strains as well as methods for detection of BMDE.

POTENTIAL MECHANISMS BY WHICH BM CELLS TAKE ON THE GENE EXPRESSION PATTERN OF EPITHELIAL CELLS

There are several possible mechanisms for BMDC plasticity. One possibility is that BM cells that differentiate into epithelial cells represent a previously unsuspected population of highly pluripotent stem cells located in the BM that have not yet “committed” to becoming blood; that is, there may be cells that have the ability to self-renew, and to differentiate into hematopoietic stem cells and into epithelial cell lineages. There have

TABLE 1. SUMMARY OF PUBLICATIONS EXAMINING BONE MARROW-DERIVED EPITHELIAL CELLS IN THE LUNG

Cells/Route	Damage Model	Findings	
MSC			
IV	Bleomycin	Type I (potential artifact)	(15)
<i>In vitro</i>	Co-culture	BMDE, fusion	(16)
IV	Bleomycin	Engraftment (epithelial-like cells) ↑ w/ Bleo, ↓ fibrosis	(17)
IV 6h p Bleo	Bleomycin ± busulfan	↑ Survival/repair ↑ G/GM-CSF ↓ Inf. cytokines	(18)
<i>In vitro</i>	Co-culture	Epithelial-like morphology, CFTR+ epithelial cells <i>in vitro</i>	(19)
IV (WBM too)	Naphthalene	Rare airway epithelial cells (CFTR+)	(20)
IT	LPS IT	↑ Survival/repair ↓ Inflammation/edema	(21)
IV	IP LPS	Prevention of LPS induced inflammation/injury/edema	(22)
Adh BM			
Adherent BM (7d)	IP Naphthalene	BM-derived epithelial cells identified after damage	(43)
WBM/HSC			
IV	Irradiation	Rare BMD epith cells	(4)
IV	LPS	GFP+ alveolar epithelial cells	(44)
IV	Irradiation	No BMDE	(41)
SP/IV	Polidocanol IT	0.83% tracheal epith.	(45)
IV (rat) BMT	Asbestos	Epithelial cells, BADJ localization	(46)
IV (human) BMT	Chemotherapy	Epithelial cells, endothelial cells	(47)
IV (SP cells)	Irradiation	Type I, but not Type II, alveolar cells, rare bronchial epith	(48)
Other			
Parabiosis	Radiation ± elastase	Type I cells	(42)
Circulating precursor	Tracheal transplant	Recipient derived epithelial cells	(40)
Type II IT	Bleomycin	Decreased fibrosis, Y+ cells localized to fibrosis	(49)
Circulating precursor	Lung transplant (human)	Epithelial cells	(50)
		Epithelial cells in bronchi, alveoli	(51)

Definition of abbreviations: AM = alveolar macrophage; BADJ = bronchoalveolar duct junction; BMDE = bone marrow derived epithelial cells; BMT = bone marrow transplantation; G/GM-CSF = granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor; HSC = hematopoietic stem cell; IP = intraperitoneal; IT = intratracheal; IV = intravenous; LPS = lipopolysaccharide; SP = side population; WBM = whole bone marrow; Y+ = Y chromosome positive.

All studies performed in mice except where indicated.

been reports from several laboratories of highly pluripotent cells derived from the BM, including Multipotent Adult Progenitor Cells (MAPC) reported by Jiang and colleagues (26), and Very Small Embryonic-Like cells (VSELs) reported by the laboratory of Dr. Mariusz Ratajczak (27, 28).

Alternatively, the cells that are responsible for BMDE are committed HSCs that can transdifferentiate. Transdifferentiation refers to the ability of one committed cell type to change its gene expression pattern to that of a completely different cell type without cell fusion. No data have been published that directly support this possibility.

A third mechanism for plasticity could be the fusion of a BM-derived cell with a nonhematopoietic cell to form a heterokaryon, thereby converting the gene expression pattern of the original BM cell to that of the fusion partner. Reprogramming of the gene expression program of fibroblasts after fusion with myoblasts to form a heterokaryons has long been known to result in the expression of muscle-specific mRNA by the fibroblast nuclei (29), and somatic cell nuclear transfer into unfertilized oocytes results in nearly complete reprogramming of somatic nuclei (30). There are elegant published data demonstrating that fusion of a macrophage, which would be BM derived, with an injured hepatocyte *in vivo* can lead to reprogramming of the macrophage nucleus so that it expresses what are considered to be liver-specific genes (31, 32). We have demonstrated that 20 to 50% of BMDE in the lung after BM transplantation are due to fusion (33). Thus, cell fusion has been proven to be at least one mechanism by which BMDE develop.

A fourth suggestion has been that a cell can acquire mRNA by taking up microvesicles containing mRNA from other cells (34). When the mRNA is released into the cytoplasm of the marrow-derived cells, one can detect not only this epithelial cell-specific mRNA, but protein translated from this mRNA (35). Uptake and expression of lung epithelial cell-derived mRNA by co-cultured BM-derived cells has been demonstrated (36). Lateral transfer of RNA has also been suggested by data obtained using xenogeneic transplantation of human BM cells into immunodeficient mice. In this work, human albumin was found in murine hepatocytes that did not contain human nuclear material, based on staining for human versus murine *alu* sequences. The authors suggest that this represents lateral gene transfer, which may have occurred when small fragments of human nuclei are retained after murine cells phagocytose deteriorating transplanted human cells (37).

A fifth possibility is that there are epithelial progenitor cells in the BM that are capable of engraftment as epithelial cells, but not as hematopoietic cells (38–40). It is also possible, and in my opinion very likely, that engraftment of BMDE occurs via multiple different mechanisms.

BRIEF OVERVIEW OF PUBLISHED LITERATURE ON BM-DERIVED LUNG EPITHELIAL CELLS

A summary of some of the published literature on BM-derived epithelial cells in the lung is shown in Table 1. (Note that this list is not exhaustive.) Studies have demonstrated the ability of MSC to take on the gene expression pattern of lung epithelial cells *in vitro* as well as after *in vivo* administration either intravenously or intratracheally. The *in vitro* studies always require co-culture of the MSC with lung epithelial cells, leaving open the possibility of microvesicles containing mRNA being taken up by the BM-derived cells, or even of contaminating mRNA in the RNA isolated from the BM-derived cells after their removal from the co-culture conditions.

In vivo studies have shown mixed results depending upon the cell population transplanted, the means of transplantation, the

type of tissue damage induced, and the methods used to detect BM-derived cells. Kotton and coworkers (15) published their finding that β -galactosidase-expressing BM cells could become type I pneumocytes after intravenous infusion into mice whose lungs had been damaged with bleomycin. Similarly, Ortiz and colleagues (17) showed that intravenous administration of MSC to busulfan-injured mice led to engraftment of epithelial-like cells and also decreased busulfan-induced fibrotic injury to the lung. However, Kotton and colleagues found no BMDE in subsequent studies when they administered BM cells into irradiated mice (41), at which time they suggested that their original identification of β -galactosidase-expressing type I pneumocytes may have been due to staining artifact rather than the true appearance of BMDE. These authors are to be commended for this reconsideration, as much of the data so far published on BMDE is dependent on morphology alone, and the staining and resolution of the imaging techniques are not always optimized for definitive identification of BMDE in lung tissue. More definitive identification approaches have involved the use of confocal microscopy and also the identification of single individual BM-derived epithelial cells analyzed after digestion and isolation of purified cells from the tissues (33), which allows one to avoid the risk of misinterpretation of the data due to one cell overlapping another.

It is both surprising and potentially clinically relevant that administration of BM cells can decrease tissue damage and/or promote tissue repair after injury even without the appearance of BMDE. Such effects have been reported, for example, after administration of either LPS or bleomycin with busulfan (17, 18, 21, 22). The mechanism(s) underlying these effects are not yet known, but *in vitro* studies strongly suggest that anti-inflammatory paracrine factors are produced when MSC are co-cultured with alveolar macrophages (21). BM-derived cells may also induce production of proangiogenic factors.

The presence of circulating epithelial progenitor cells, which may or may not be derived from the bone marrow, is suggested by studies using multiple different approaches. When two mice were surgically linked side by side so that they had shared circulatory systems, a process called Parabiosis, and the lung tissue of one of the mice was injured by irradiation with or without elastase, the healing lung tissue contained type I pneumocytes that had been derived from the uninjured mouse (42). Similarly, when a denuded trachea was transplanted into a mouse, the epithelial cells that grew in the ectopically transplanted trachea were derived from the recipient (40). Whether the circulating cells are true progenitor cells or differentiated epithelial cells is not yet known. Intratracheal transplantation of mature type II pneumocytes.

CONCLUSIONS

BM-derived cells can take on the gene expression profile of lung epithelial cells both *in vitro* and *in vivo*. The mechanisms underlying this change in gene expression pattern are not yet known, nor is it known which BM populations are responsible. What we do know so far is that multiple different BM-derived cell populations, including marrow stromal cells (aka mesenchymal stem cells) and hematopoietic cells, are capable of being reprogrammed. Regarding the mechanism, some BM-derived epithelial cells form by cell–cell fusion, which presumably involves fusion of a BM-derived blood cell such as a macrophage with an injured epithelial cell; while other BMDE do not show evidence of having been derived from cell fusion. Although there is still much research to be done, a better understanding of the reprogramming of BM-derived cells may lead to discovery of novel pathologic processes and/or clinically relevant approaches to promoting tissue repair.

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