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Michael R. Loebinger and Sam M. Janes

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Stem Cells for Lung Disease*

Michael R. Loebinger, BM, BCh, MA (Hons); and Sam M. Janes, MSc, PhD

Respiratory diseases remain one of the main causes of morbidity and mortality in the world. Interest has increased as to the possibility of optimizing the repair of the lung with the manipulation of stem cells. Embryonic and adult stem cells have been suggested as possibilities. Adult stem cells have traditionally been thought of as having limited differentiation ability and to be organ specific. However, a series of exciting reports over the last 5 to 10 years have suggested that adult bone marrow-derived stem cells may have more plasticity and are able to differentiate into bronchial and alveolar epithelium, vascular endothelium, and interstitial cell types, making them prime candidates for repair. This article critically reviews the evidence for this plasticity and the use of predominantly adult stem cells to help with lung regeneration and repair and assesses how this technology may be utilized in clinical medicine.

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Key words: bone marrow; endothelium; lung; progenitor; repair; respiratory; stem cell; therapy

Abbreviations: CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; EPC = endothelial progenitor cell; GFP = green fluorescent protein; HSC = hematopoietic stem cell; MSC = mesenchymal stem cell

The lung is a complex organ with limited regenerative capacity. Local stem cells, with the capacity for unlimited self renewal and the production of more committed progenitors, have long been thought of as central to the repair and regeneration processes of various organs. These reparative processes have been well described in organs such as skin; however, the endogenous stem cells of the respiratory tract have not been fully elucidated. Studies[1] of epithelial damage in animal models suggest different local stem populations situated throughout the tract, with basal epithelial cells in the trachea and larger airways, and Clara cell secretory protein-expressing cells the likely candidates in the smaller airways. Type 2 pneumocytes are thought to act as the main local endogenous stem cells of the lung parenchyma. Side population (CD45-negative, Hoescht effluxing) cells have also been identified in the lung, but their role in endogenous repair is not clear.[2] Nevertheless, whatever the dominant population, this endogenous repair is insufficient to prevent the many progressive respiratory diseases.[3] Ongoing research is looking at the manipulation of stem cells and specifically the use of extrinsic stem cells to augment lung repair to improve the response of the lung parenchyma to injury and disease, and this is the focus of this review

The main body of work to date has centered on the use of adult stem cells. Adult stem cells are classically limited to differentiating into cell types of their tissue of origin (Table 1). This viewpoint has recently been reassessed, as many studies[4–14] have demonstrated a degree of plasticity of adult bone marrow stem cells, with an apparent ability to cross lineage barriers, adopting functional phenotypes of other tissues. This has led to research assessing the potential of adult stem cells in the treatment of various disorders. A potential advantage of using stem cells from an adult is that the patient's own cells could be expanded in

*From the Centre of Respiratory Research, University College London, London, UK.
Dr. Loebinger is a Medical Research Council clinical training fellow. Dr. Janes is a Medical Research Council clinician scientist.
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Correspondence to: Sam M. Janes, MSc, PhD, MRCP, Centre of Respiratory Research, Rayne Building, University College London, 5 University St, London, WC1E 6JJ, UK; e-mail: s.janes@ucl.ac.uk
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Table 1—*Glossary of Commonly Used Terms*

Stem cell	Cells that have unlimited self-renewal properties and also have the ability to produce more differentiated daughter cells
Progenitor cell	Cells able to divide and further differentiate
Transit-amplifying cell	The progeny of stem cells with the ability to proliferate and differentiate
Plasticity	Ability of cells to cross lineage barriers adopting phenotypes of other tissues
Self renewal	Ability of cells to produce at least one identical daughter cell during cell division
Totipotent	Ability to produce all cells of organism
Pluripotent	Ability to produce cells of all lineages
Multipotent	Ability to produce cells of multiple lineages
Unipotent	Ability to produce cells of a single lineage

culture and then reintroduced into the patient. The use of autologous cells would help avoid some of the problems associated with immunologic rejection.

Plasticity of Adult Bone Marrow-Derived Stem Cells

Bone marrow contains hematopoietic stem cells (HSCs), which characteristically differentiate into every type of mature blood cell, and mesenchymal stem cells (MSCs), which differentiate into fat, bone, cartilage, and other mesenchymal tissues.[4] Many studies[5–14] have shown that cells derived from adult bone marrow are able to produce a variety of nonhematopoietic cells both *in vitro* and *in vivo*. The *in vivo* studies have mostly used systemic infusions of bone marrow stem cells into recipient mice. The donor cells are lineage marked and distinguished from host cells either phenotypically (*eg*, expressing green fluorescent protein [GFP]), or genotypically (*eg*, containing the Y chromosome). The fate of the donor-derived cells is then assessed in several organs by histology, immunohistochemistry, and functional assays.[5–14]

An early study[5] demonstrating the contribution of lung tissue by bone marrow stem cells was published in 2001. In this study, a single HSC was transplanted from an adult male mouse into a female that had been lethally irradiated to ablate the resident bone marrow. This single donor cell was able to repopulate all bone marrow cell lineages but also engrafted several organs. Up to 20% of the lung parenchyma in the donor mouse was found to contain a Y chromosome, which colocalized with epithelial markers.[5] Similar animal studies[6–14] showing varying levels of engraftment of bone marrow stem cells as alveolar and airway epithelial tissue ensued. Models in human beings were also provided by sex-mismatched transplant patients. Female recipients of male bone marrow were shown to have pulmonary chimerism, with male donor cells engrafted as epithelial and endothelial cells.[15] This finding was also repeated in male recipients of a female lung allograft. In this study,[16] quantitative analysis revealed that the greater levels of engraftment occurred at sites of greatest injury following rejection or infection. This observation was duplicated in the animal experiments, whereby there appeared to be a greater amount of engraftment to the lung if the parenchyma was injured before transplantation (*eg*, by radiation or bleomycin injury).[6,9–14,17,18] The suggestion from these studies is that the bone marrow stem cells were actively recruited by injured lung to help with the repair process.

Lung engraftment, however, has not been confirmed in all studies, and more recently the area and methodology have been reevaluated. It is now accepted that previously used methods of detecting engraftment have not been sufficiently rigorous (Table 2), particularly with the problems of donor-derived inflammatory cells migrating to areas of damage. Techniques used to assess lung chimerism as evidence for engraftment (particularly immunohistochemistry) have been shown to be subject to significant artifacts leading to an overestimation of the amount of engraftment.[19,20] One study[19] employed a lineage-specific reporter system based on transgenic mice that express the GFP reporter gene only in lung epithelial cells (surfactant protein-C–GFP) to assay for engrafted cells by flow cytometry, histology, and molecular methods. This showed no evidence of bone marrow-derived epithelial cells.[19] This conclusion was also reached by a related article[20] that exposed apparently chimeric lung cells as overlapping cells with deconvolution microscopy. Some of the discrepancy and lack of consistency between study outcomes is also attributable to study design. In each experiment, different stem cells have been used as donors (*eg*, MSCs, side population stem cells, HSCs, whole bone marrow, endothelial progenitor cells [EPCs], multipotent adult progenitor cells[21]), and the experimental conditions and recipients have differed.

The present consensus, including the authors of the original articles, suggest that engraftment of

Table 2—*Commonly Used Techniques To Detect Engraftment of Stem Cells

Techniques	Advantages	Problems	Reference No.
Light immunohistochemistry	Morphologic image of labeled cells	Subjective Nonspecific antibodies; overlapping cells	5–21,31,32,37
Fluorescent immunohistochemistry	Dual staining of donor label and marker for engrafted cell phenotype	Subjective; nonspecific antibodies; overlapping cells; autofluorescence from apoptotic cells	5–21,31,32,37
Flow cytometry	Removes problems of overlapping cell artifact	No morphologic information; fixation and staining for intracellular epitopes eliminates ability to exclude dead cells and increases background; nonspecific antibodies	11,19,20
PCR or RT-PCR of tissue	Sensitivity	Will include circulating cells	7,12,19,37
Laser capture microdissection and RT-PCR or PCR	Removes problems of nonspecific staining and circulating cells	No morphologic information	16
Confocal/deconvolution immunohistochemistry	Remove problems of overlapping cell artifact	Autofluorescence; nonspecific antibodies	8,14,20,37
Reporter genes under cell-specific promoter	Cells only labeled if change lineage	Misses engraftment without activation of promoter	19,20

*PCR = polymerase chain reaction; RT-PCR = real-time polymerase chain reaction.

bone marrow-derived stem cells as airway and alveolar epithelium is likely to occur but a very low rate (0.01 to 0.1%) of doubtful clinical significance.[22] Future studies should use multiple methods of detection and state of the art techniques including deconvolution microscopy, laser-capture microdissection, and flow cytometry to adequately and unambiguously define possible engraftment and functional data, should be available where possible.

In contrast to engraftment and repair of the respiratory epithelium, there is accumulating evidence for the contribution of bone marrow-derived stem cells to the fibroblast/myofibroblast community in the lungs via circulating fibrocyte cells.[11,17,18] Fibrocytes are circulating cells expressing the HSC marker CD34 and collagen1. In fact, 80% of type 1 collagen-expressing fibroblasts were shown to be of bone marrow (donor) origin at sites of lung fibrosis in a mouse bleomycin model.[11] Labeled fibrocytes have also been shown to be recruited into bronchial tissue and differentiate into myofibroblasts following allergen exposure in an animal asthma model, and fibrocyte-like cells have been detected in human bronchial biopsy specimens.[23] There is also convincing evidence for engraftment into pulmonary vasculature by other bone marrow progenitors; endothelial progenitor cells. EPCs have been isolated from the bone marrow of rats and shown to express endothelial markers and engraft into areas of vascular injury in animal models of pulmonary hypertension.[24]

Migration, Targeting, and Engraftment

The production of substances chemotactic to circulating stem cells by injured lung has been shown in an *in vitro* experiment[13] whereby bone marrow-derived MSCs, cocultured with injured lung, showed a marked proliferation and migration that was not apparent when noninjured lung was used. Injured lung is thought to produce several chemokines, including hyaluronan, osteopontin, stromal-derived factor 1α, and secondary lymphoid chemokine, which interact with several receptors present on bone marrow-derived cells including CD44 on MSCs,[12] and CXCR4 and CCR7 on bone marrow-derived fibrocytes.[11,25] The importance of this migration and targeting process has also been demonstrated by blocking chemokines with specific antibodies, which reduced the bone marrow contribution to lung fibroblasts, as described later.[25]

The mechanism of phenotypic conversion of bone-marrow derived cells has not been determined. Fusion of the bone marrow progenitors with existing lung cells was suggested as a possible cause for the observed plasticity and colocalization of markers. Although this mechanism is thought to be important in other organ systems,[26] it has not been shown to be important in the lung.[27] It is likely that any phenotypic conversion is mediated by soluble factors and direct cell-cell contact.

Effects of Stem Cell Engraftment in the Lung

Bone marrow-derived stem cells do appear to be recruited to lung tissue as described in the above experiments. The effects of this process on the lung tissue and its response to injury are discussed below.

Two studies[9,13] attempted to show that the normal repair of the lung relied on repair by circulating stem cells (from the bone marrow) as well as local, endogenous repair processes. They showed that lung

damage secondary to lipopolysaccharide or bleomycin was increased if the bone marrow response was suppressed by either radiation or busulphan and the mortality of the mice was increased in the latter experiment. This could be reversed if bone marrow stem cells were transplanted. Studies[10,12,13] have demonstrated that if the process of stem cell migration was augmented, lung injury could be reduced. Intraperitoneal injection of all-trans retinoic acid or granulocyte colony stimulating factor increased bone marrow stem cell engraftment in the lungs of mice after intranasal elastase had induced emphysema. In both cases, the extent of emphysema was consequently reduced compared to mice that had been injected with a control substance.[10] Direct additional bone marrow stem cell administration has also been shown to reduce damage in injury models. MSC administration immediately after exposure to bleomycin in mice was associated with a significant reduction in bleomycin-induced inflammation and collagen deposition within lung tissue.[12,13] The rates of engraftment in these studies were very small, and it is likely that mechanism of improvement is mostly due not to stem cell engraftment of the injured tissue, but the effects of paracrine secretion of growth factors and cytokines stimulating repair.

EPCs have been shown to prevent progression of pulmonary arterial hypertension in a rat model, by engrafting the arteriolar bed.[24] As previously, the relative effects of cytokines and cellular repair have not been extracted. EPCs have also been implicated in repair in human studies,[28,29] in which increased circulating EPCs are associated with improved outcomes from acute lung injury and bacterial pneumonia.

Conversely, other authors have suggested that bone marrow-derived stem cells can actually have a negative impact on functional recovery of the lung to injury. As described earlier,[11] there was a bone marrow-derived contribution of fibroblasts at sites of lung fibrosis in a mouse bleomycin model. Similarly, bone marrow-derived fibroblasts proliferated and contributed to lung fibrosis after homing in response to the chemokine CXCL12. The fibrosis in this latter experiment[25] could then be reduced using CXCL12 antibodies. Circulating fibrocytes have also been implicated in contributing to subepithelial fibrosis of the airways in animal models of asthma[23]

It is important to consider the role that stem cells may have in the development of cancer. The same properties of unlimited self renewal that make stem cells attractive agents for repair also make them candidates for uncontrolled growth and malignant transformation. An elegant experiment highlighted this potential in a mouse model of gastric cancer. In this model C57BL mice were myeloablated and transplanted with gender-mismatched, GFP-labeled bone marrow. The mice were then infected with *Helicobacter felis*, which leads to chronic inflammation and gastric carcinoma. In this model, the neoplasia arose from donor bone marrow cells, strongly suggesting an inherent vulnerability of this population to malignant transformation.[30] Furthermore, in other experiments bone marrow stem cells have also been shown to contribute to myofibroblasts and fibroblasts of tumor stoma.[31]

It appears clear that different experimental variables, donor stem cells, and recipients can alter the engraftment process and response of the lung to injury significantly. Nevertheless, the prospect of being able to alter repair and the response to injury using this mechanism is extremely attractive.

Therapeutic Possibilities

Stem cells have a multitude of clinical implications in the lung[32,33] (Fig 1). The realization that adult bone marrow stem cells may contribute to the repair of lung injury allows this avenue to be manipulated and augmented in human diseases. Treatment by cell therapy could be envisaged for acute disorders such as ARDS, or more chronic disorders such as emphysema and lung fibrosis, as suggested by the murine models described above. Nevertheless, the consensus is now that respiratory epithelial engraftment is a very rare event, and as such cellular repair is not thought to be realistic at present.

Engraftment of stem cells can also be used for genetic therapy. In a mouse model,[34] bone marrow stem cells were genetically altered with a viral vector to express GFP. These transplanted cells then engrafted in the recipient lung, differentiating into lung epithelium while maintaining long-term transgene expression.[34] Stem cells may therefore act as genetic vectors in certain diseases needing replacement of a protein or DNA. A striking example of this demonstrated that a mouse with fatal genetic tyrosinemia (fumaryl acetoacetate hydrolase deficient) could be cured with the transplantation of wild-type bone marrow stem cells, which engrafted in the liver and produced the necessary protein product.[35] Within respiratory medicine, cystic fibrosis (CF) is a devastating illness that may similarly benefit from such an approach. A study[36] providing further encouragement used genetic transduction of bone marrow stem cells *ex vivo* from CF patients to enable them to express the normal CF transmembrane conductance regulator (CFTR). These cells were then mixed in a human airway-epithelial culture with epithelial cells from CF patients. The stem cells were able to differentiate into airway epithelia cells and partially

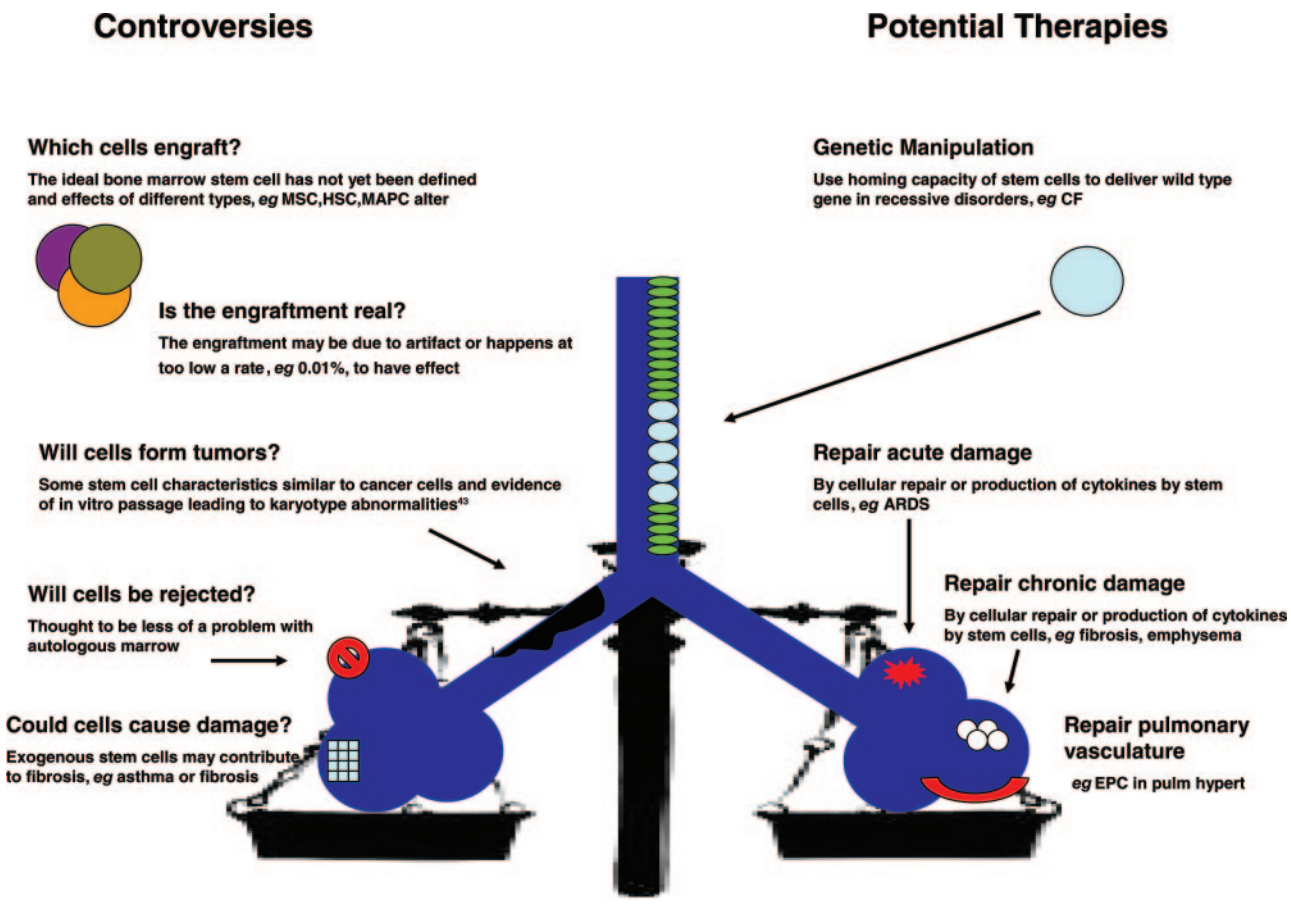

FIGURE 1. The controversies and potential therapeutic uses of stem cells in lung disease. pulm hypert = pulmonary hypertension.

correct the defective CFTR-dependent chloride current.[36] *In vivo*, wild-type bone marrow cells were able to engraft in the lungs of CFTR knockout mice and acquire epithelial phenotypes including the expression of CFTR messenger RNA and protein. However, this was an extremely rare event (0.01%) and unlikely to be of any functional significance, as approximately 10 to 15% correction of defective airway epithelium has been estimated to be needed to allow normal chloride current, thus emphasizing the gap between proof of concept data and clinical use.[37]

More encouragingly, gene therapy has been used successfully in the monocrotaline-induced pulmonary arterial hypertension model, whereby rats were administered EPCs transduced with human endothelial nitric oxide synthase, which reversed the established disease and significantly improved survival.[24] As a result of this, a phase 1 safety trial (Pulmonary Hypertension: Assessment of Cell Therapy) has just been commenced in Canada, whereby patients with refractory pulmonary hypertension are receiving autologous EPCs transfected with endothelial nitric oxide synthase via a pulmonary artery catheter.

As well as cellular and genetic therapy for lung diseases, an appreciation of stem cells may improve the understanding and treatment of certain malignancies. There is an increasing belief that cancers contain a small number of cancer stem cells and that the majority of cells within the tumor are not capable of tumor initiation. Furthermore, these cancer stem cells are often relatively quiescent, suggesting that conventional antiproliferative chemotherapy agents may spare these cells, leading to tumor recurrence. A greater understanding of the role of cancer stem cells may help to develop specific therapies targeting them and not the rapidly proliferating daughter cells. In this instance, the detection of cancer stem cell-specific surface markers or unique molecular targets would allow directed molecular therapy.[38]

EMBRYONIC STEM CELLS

Embryonic stem cells are cultured from the developing blastocyst and are pluripotent with the ability to differentiate into all the cells of the body.

At present, use of embryonic stem cells in lung disease is lagging behind that of adult stem cells. The potential of these cells can be demonstrated by articles[39] describing the generation of airway and parenchymal epithelium *in vitro*. There have been concerns, however, with the potential for malignant transformation and immune rejection in hosts.[40] Furthermore, research with these cells involves the destruction of an embryo, and as such has met with moral and ethical objections. There is also a strong political input particularly in America, where there remain substantial prohibitions on the use of embryonic stem cells.

Benchside to Bedside and Back Again

Successful cellular or genetic therapy with stem cells will require specific targeting to the appropriate site and long-term engraftment. Expression of the correct phenotype and function and expression of manipulated genes will also need to be tightly controlled, and there will need to be a lack of malignant transformation in these treatments. Importantly, an appreciation of the most appropriate stem cell type to be transplanted will be needed. There is great excitement and potential with these therapies, but at present the field is littered with controversies and questions (Fig 1). Although we are some way off from clinical trials with bone marrow stem cells in parenchymal lung disease until some of the above issues are clarified, such is the promise of this therapy that it has already been used in human cardiac trials. Clinical trials[41] in cardiology have demonstrated the feasibility and safety of administering progenitor cells derived from autologous bone marrow to the myocardium of patients with ischemic heart disease. The few controlled trials that have been completed show a tendency to improved heart function in transplanted patients both in the acute infarcted heart, and in those with chronic ischemic heart failure.[41] Nevertheless, in view of the above uncertainties, significant concern has been raised as regards the wisdom of clinical trials at present.[42] Although stem cell therapy in the lung has great promise, further basic science research is needed before this treatment can be confidently moved to the bedside.

Conclusion

There is evidence that bone marrow-derived stem cells are able to target areas of the body undergoing injury and contribute to repair. The exact mechanisms are not wholly understood, and different cell species may have different roles in certain situations. Nevertheless, this basic science research is likely to open up new and exciting avenues of pharmacologic, cellular, and genetic therapy for diseases in which they are greatly needed—no more so than in respiratory medicine.

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