

## CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor***Pulmonary Alveolar Proteinosis and Macrophage Transplantation**

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Macrophages are highly variable, and their ontogeny and functions depend on the particular organ in which they reside and on any ongoing disease processes. Lung macrophages are critically important to lung function, and knowledge of their ontogeny and phenotypes is rapidly expanding. Lung macrophages are present within the alveolar spaces and in the airway wall and lumen. Airway macrophages represent both alveolar macrophages that are on their way along the mucociliary escalator toward the nasopharynx and other distinct populations of macrophages, which may have their own ontogeny and function. In healthy lungs, the overarching functions of macrophages include serving as a host-defense surveillance system that protects the lungs, modulating (often dampening) inflammatory and immune responses, and monitoring and regulating the surfactant layer, which is critical to maintaining a low surface tension and enabling the opening of alveoli with each breath.

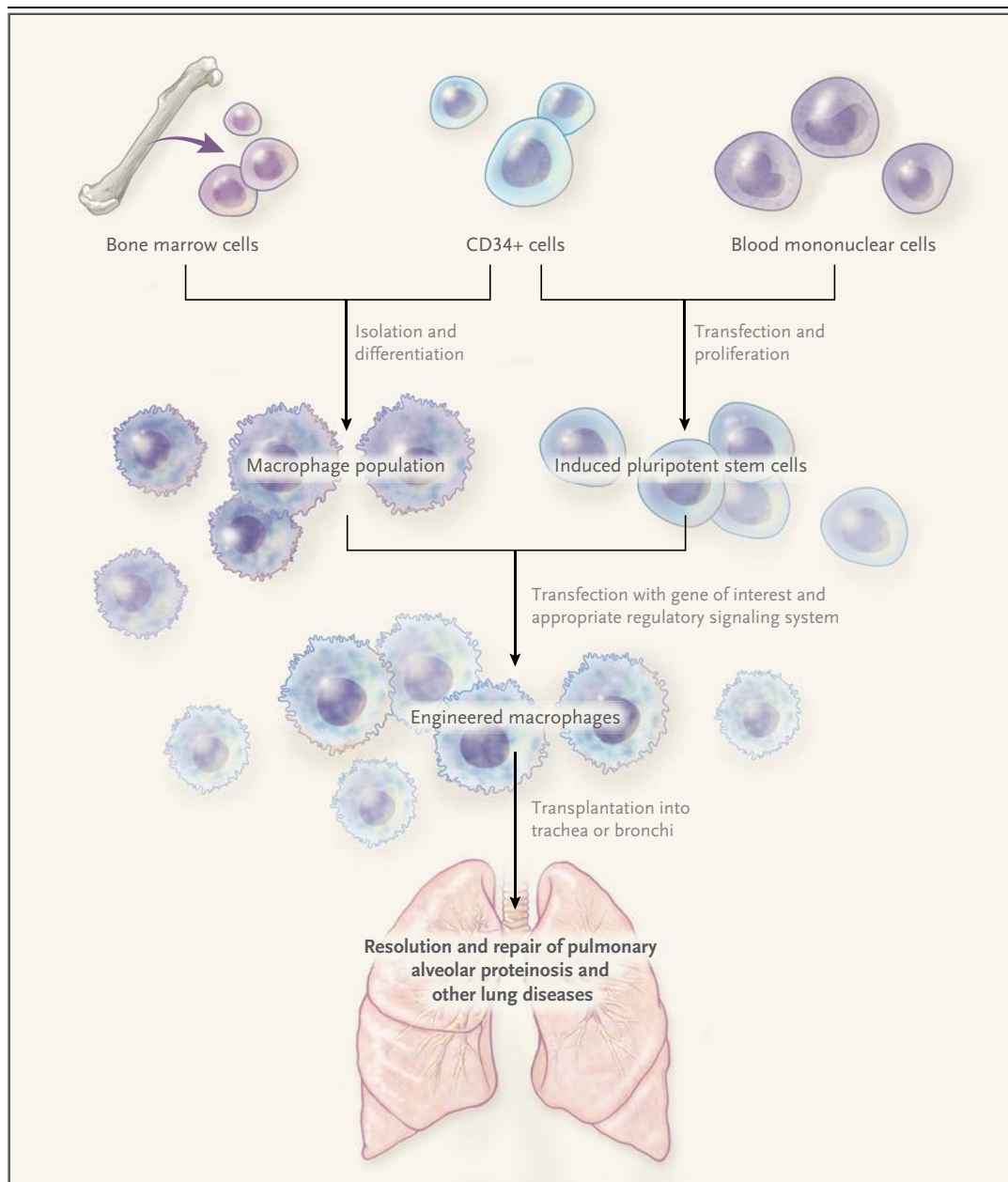
In disease, lung macrophages play widely varied roles. They can initiate inflammatory responses to gaseous, particulate, and microbial threats, facilitate the resolution of inflammatory and innate immune reactions, and participate in fibrotic or granulomatous reactions that result in alveolar or airway remodeling and defects in lung function.

The alveolar surfactant lining layer is tightly and continuously regulated by means of the production of surfactant phospholipids and proteins by the type II alveolar epithelial cells and by means of the clearance of ineffective, often oxidized, surfactant by the alveolar macrophages. Cell signaling initiated by the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor is critical to the breakdown of surfactant by macrophages. Pulmonary alveolar proteinosis is caused by defective signaling by the GM-CSF receptor,

usually owing to a genetic deficiency that results in the diminished expression or function of the  $\alpha$  or  $\beta$  subunits of the GM-CSF receptor or, more commonly, to autoantibodies against GM-CSF, resulting in defective clearance of surfactant lipoprotein from the alveolar space. The distal lung tissue becomes filled with many massively enlarged alveolar macrophages that are able to ingest surfactant but unable to process it. Ultimately, decreased gaseous exchange results in hypoxemic respiratory failure and defects in host defense. The human disease is closely mimicked — both structurally and physiologically — by deficiency of the GM-CSF receptor in mice, a disease model obtained by means of knockout of *Csf2rb*, which encodes the  $\beta$  subunit of GM-CSF.

Kleff and colleagues<sup>1</sup> found that reconstituting the bone marrow of these mice with murine *Csf2rb*<sup>-/-</sup> hematopoietic stem cells that were genetically engineered to express the mouse GM-CSF receptor completely reversed the lung pathology of surfactant accumulation within 12 weeks, despite modest transduction levels of 10 to 20% (i.e., only a small fraction of the donor cells expressed the construct). Pulmonary alveolar proteinosis developed in mice that were engineered to replace the murine GM-CSF protein with the human GM-CSF protein, because human GM-CSF is not recognized by the mouse GM-CSF receptor. The phenotype of this model of pulmonary alveolar proteinosis was ameliorated when the bone marrow of mice was reconstituted with human CD34+ cells (from which macrophages differentiate).<sup>2</sup> These studies show that replacement with functional bone marrow-derived macrophages restores surfactant homeostasis. But in humans, the morbidity and mortality of bone marrow ablation and transplantation limit their usefulness.

The possibility of transplanting macrophages,



**Figure 1. A Potential Path to Pulmonary Macrophage Transplantation.**

A schema for an approach to pulmonary macrophage transplantation in humans is suggested by several studies of mouse models of pulmonary alveolar proteinosis. It involves the isolation and differentiation of macrophages from CD34+ bone marrow cells. Alternatively, induced pluripotent stem cells could be generated from blood mononuclear cells or CD34+ bone marrow cells. These macrophages or induced pluripotent stem cells could then be transfected with the gene or genes of interest and an appropriate regulatory signaling system. In the case of pulmonary alveolar proteinosis, transfection with *CSF2RB* (encoding the granulocyte–macrophage colony-stimulating factor [GM-CSF] receptor) would probably be most effective. These macrophages could then be transplanted into the trachea or proximal or distal bronchi by means of bronchoscopic visualization of the airways and instillation. Effective treatment would depend on engraftment of these modified cells.

perhaps after genetically manipulating them, by directly introducing them into a patient's lungs and thus effecting repair of lung injury has long been a goal. A combined gene-and-cell approach has the potential to benefit patients with a wide range of lung diseases, including hereditary pulmonary alveolar proteinosis (Fig. 1). Three studies bring this goal closer, at least for patients with pulmonary alveolar proteinosis. Happle et al.<sup>3</sup> transplanted bone marrow–derived macrophage progenitors from wild-type mice into the airways of *Csf2rb*<sup>-/-</sup> mice and found that these progenitors abrogated the mice's phenotype for pulmonary alveolar proteinosis and were detected only in the lungs from 5 weeks to 9 months after transplantation. Second, the other model of pulmonary alveolar proteinosis, humanized mice expressing the human interleukin-3 and GM-CSF genes, was effectively treated by intrapulmonary transplantation of cells derived from CD34+ cells isolated from human cord blood.

Third, Suzuki et al.<sup>4</sup> found that the transplantation of bone marrow–derived, wild-type, highly purified, mature macrophages into the lungs of *Csf2rb*<sup>-/-</sup> mice resulted in proliferation of these macrophages, replacement of the *Csf2rb*<sup>-/-</sup> alveolar macrophages, and reversal of the lung disease. Moreover, the progeny of these macrophages were present in the lungs 1 year after transplantation. Suzuki et al. also found that the transplantation of bone marrow–derived *Csf2rb*<sup>-/-</sup> macrophages corrected by lentivirus-mediated *Csf2rb* complementary DNA expression abrogated pulmonary alveolar proteinosis in *Csf2rb*<sup>-/-</sup> mice. Both wild-type and *Csf2rb*-corrected macrophages lost features of bone marrow macrophages and gained those of tissue-resident lung macrophages, a finding that is consistent with previous observations that the lung microenvironment is critical in determining the phenotype and function of macrophages. These studies suggest that autologous, gene-corrected, bone marrow–derived macrophages represent a potential therapeutic intervention for patients with the hereditary form of pulmonary alveolar proteinosis. Lachmann and colleagues have already generated patient-specific, gene-corrected, induced pluripotent stem cells.<sup>5</sup>

A clinical trial of pulmonary macrophage transplantation in patients with pulmonary alveolar proteinosis is warranted. There are many possibilities for this combined cell–gene therapy to

benefit our patients. Macrophages that are genetically engineered to regulate oxidative stress or enzymatic degradation may serve therapeutic purposes. One might envision the transplantation of macrophages that express genes encoding proteins that down-regulate or even break down fibrotic tissue in diseases such as interstitial lung disease or pulmonary fibrosis. Macrophages that are engineered to inhibit proteases or to secrete matrix materials or building blocks may be valuable in diseases such as emphysema. Macrophages that possess ion channels that can modulate the hydration of airway liquid may be helpful in the many common diseases (e.g., chronic bronchitis and cystic fibrosis) in which abnormal viscoelastic properties of sputum alter epithelial responses and host defense. The current focus on airway exosomes (minute, cell-derived vesicles that can transfer genes and proteins from one cell type to another) brings to mind the possibility that macrophages that have been engineered to express the messenger RNA of the gene encoding the cystic fibrosis transmembrane conductance regulator (*CFTR*) could be used to treat patients with cystic fibrosis. All that being said, caution is, of course, needed: the long lifetime of these macrophages and their ability to proliferate and alter lung homeostasis raise concerns about safety. In sum, the opportunities for pulmonary macrophage transplantation are many and are worthy of further exploration.

Disclosure forms provided by the author are available with the full text of this article at [NEJM.org](http://NEJM.org).

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