look at the length of how long these effects last and the cells won't be there?

Dr Hwang. That would be the next step. We would do some survival studies to see how long these effects would be, and then eventually we would like to go into more of

a larger animal model, dog studies, which we have a lot of experience in, and take those lungs that have been conditioned with the special cocktail and use those in a 1-transplant setting to see if that maintains some level of tolerance long-term.

EDITORIAL COMMENTARY

How do cells talk to each other?: Paracrine factors secreted by mesenchymal stromal cells

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Mesenchymal stromal cells (MSCs) are the subject of several clinical trials. In parallel with their major role as undifferentiated cell reserves, MSCs have immunomodulatory functions that are exerted by direct cell-to-cell contacts, by secretion of cytokines, and/or by a combination of these. Early hypotheses suggested that MSCs caused cell replacement and differentiation as their main therapeutic mechanism of action.

Evidence is mounting that secretions of MSCs are responsible for their therapeutic effects. These secretions include molecules and extracellular vesicles that have both local and distant effects—loosely referred to as “paracrine” properties. Paracrine signaling is a form of cell-to-cell communication in which a cell produces a signal to induce changes in nearby cells, altering the behavior or differentiation of those cells. Paracrine factors secreted by stem cells can do the following: induce surrounding cells to differentiate into mature cell lines; modulate inflammatory or reparative processes in surrounding tissues; and play an important role in the actions of the stem cells that secrete them, particularly MSCs. These factors allow 1-way conversations between stem cells and more-differentiated cells.

The bone marrow contains many types of cells. Among them are blood stem cells (also called hematopoietic stem cells [HSCs]) and various types of stem

cells belonging to a group called mesenchymal cells. Only about 0.01% of the cells in the bone marrow are MSCs. Obtaining a mixture of mesenchymal cell types from adult bone marrow, for research, is fairly easy. But isolating the tiny fraction of cells that are MSCs is more complicated. Some cell clones in the mixture may be able to form bone or fat tissues, for example, but still do not have all the necessary properties to be called MSCs.

The challenge is to identify and pick out the cells that can both self-renew (produce more of themselves) and differentiate into 3 cell types—bone, cartilage, and fat. Multiple studies in humans and rodents converged to identify the nonhematopoietic stem cells in the bone marrow (widely called “mesenchymal” stromal cells or “skeletal” stem cells). The term “skeletal” implies that these stem cells can differentiate into tissues that together make the skeleton (bone, cartilage, and fat). Some confusion occurred about types and origins of various stem cells.

Immunomodulation of mesenchymal stem cells.

Central Message

Paracrine factors secreted by mesenchymal stem cells have important physiologic effects on inflammation.

See Article page 841.

Mesenchymal Stem Cell Differentiation Pathways & Lineage-specific Markers

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FIGURE 1. Mesenchymal stem cell differentiation pathways and lineage-specific markers. *BMP*, Bone morphogenetic protein; *FGF*, fibroblast growth factor; *TGF*, transforming growth factor; *NCAM*, neural cell adhesion molecule; *PTH*, parathyroid hormone; *PDGF*, platelet-derived growth factor; *IGF*, insulin-like growth factor.

To begin to address this confusion, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed minimal criteria to define human MSCs. First, MSCs must be able to adhere to plastic when maintained in standard culture conditions. Second, MSCs must express surface markers like CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14, CD11b, CD79 α , CD19, and HLA-DR surface molecules typical of hematopoietic stem cells. Third, MSCs must differentiate to osteoblasts, adipocytes, or chondroblasts in vitro.¹

Mesenchymal stromal cells are an example of mature adult stem cells that are “multipotent,” meaning they can produce more than 1 type of specialized cells in the body, but not all types (Figure 1). They differentiate into the various specialized cells found in skeletal tissues. These specialized differentiated cells each have their own characteristic shapes, structures, and functions, and each

is associated with a particular tissue. They are found in bone marrow, but may exist in a wide variety of other tissues, such as umbilical cord blood, adipose tissue, and muscle.^{2,3} The mechanisms by which MSCs alter nearby surrounding cells is, in part, related to paracrine factors secreted by the parent MSCs.

The article by Hwang and colleagues⁴ describes their work with cell-free media from MSCs.⁴ These authors harvested rat MSCs using the properties of adherence to plastic to separate cellular components of mouse bone marrow. The culture media from these plastic-adherent cells contain paracrine factors that are specific products produced by the various MSC clones. They used cell-free media from MSCs in a rat model of lung ischemia and reperfusion and examined media from various clones of MSCs. They found that this cell-free media provided protection from lung damage induced by ischemia and reperfusion, significantly more than the MSCs themselves. They characterized

the changes induced by paracrine mediators that are associated with this protective effect of cell-free media from MSCs. They suggest that cell-free media from MSCs confer pulmonary ischemic tolerance.

Studies suggest that the capacity of MSCs to secrete soluble factors that alter the tissue microenvironment may play a more prominent role than their cellular trans-differentiation in effecting tissue repair.^{2,3} This article⁴ supports this notion. The authors present preliminary experiments in a rat model of lung ischemia and reperfusion that suggest that several clones of MSCs have the ability to produce paracrine factors that are protective against some markers of ischemia and reperfusion. One might ask many questions about the implications of this research.

Some of these questions are as follows:

- What paracrine factors in the conditioned media are responsible for the observed protective effect?
- What is different about the conditioned media from the various clones of MSCs that did not provide a protective effect in the ischemia/reperfusion model, compared with media from clones that did?
- Why don't MSCs provide the same protective effect in this model of ischemia/reperfusion as the conditioned media do?
- How were the MSC clones chosen for study? How well were the MSC clones characterized? Did all the clones have equivalent differentiation potential, despite differences in the modulatory effects of their conditioned media?
- Transplanted allogeneic MSCs can be detected in recipients at extended time points, indicating a lack of immune recognition and clearance by the host. By using conditioned media, the model chosen by the authors must of necessity be a short-term induction of immune tolerance. This induction is quite different than immune modulation induced by MSCs alone. Is the benefit from

conditioned media a short-term phenomenon that may not persist?

I realize that the authors⁴ presented interesting preliminary results and that more studies are almost certainly in progress. Some of the above questions do not have answers based on the authors' current study, but the reader must see the potential benefit that cell-free media from MSCs may offer. The authors provide provocative preliminary results in an animal model of ischemia and reperfusion that suggest an important role for paracrine secretions of MSCs in at least a lung model of ischemia and reperfusion, but more widely, in multiple physiologic processes.

This work certainly serves as a foundation for further research. The potential applications of this research are wide ranging and include the following: modulation of transplant rejection; limitation of inflammation in autoimmune disease; enhancement of regeneration of stromal cells (cartilage and bone) in orthopedic degenerative disease; limitation of graft-versus-host disease after bone marrow transplantation; induction of angiogenesis in myocardial infarction⁵; and attenuation of epithelial injury and lung fibrosis.⁶ This article⁴ presents very preliminary, though exciting, observations, and more results will surely follow from these authors.

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