

OCTOBER 12, 2003

Doubt Cast on Adult Stem-Cell Plasticity Studies

In a study that calls into question the plasticity of adult stem cells, Howard Hughes Medical Institute (HHMI) researchers and colleagues at the University of California, San Francisco, have demonstrated that adult bone marrow cells can fuse with brain, heart and liver cells in the body.

The phenomenon of fusion would give the appearance that bone marrow stem cells are altering themselves to become mature cells in other tissues, when in fact they are not, according to one of the study's senior authors, HHMI investigator Sean J. Morrison at the University of Michigan.

"Scientists should exercise caution in using
adult bone marrow cells in clinical trials
designed to generate new cells in other tissues."

— Sean J. Morrison

The researchers published their findings October 12, 2003, in the online version of the journal *Nature*. The studies were carried out by collaborating scientists, Manuel Alvarez-Dolado and Ricardo Pardo, in the laboratories of Arturo Alvarez-Buylla of the University of California, San Francisco and Morrison at the University of Michigan. Other co-authors are from the University of Valencia in Spain, the University of Dusseldorf in Germany and MIT.

The ability of bone marrow cells to contribute at very low levels to other tissues was previously interpreted as indicating that these cells had the plasticity to make new cells in other tissues. As a result, clinical trials have been initiated in which bone marrow cells have been injected into heart muscle in an effort to stimulate the formation of new heart muscle cells after heart attack.

The new findings indicate that bone marrow contributes to other tissues by fusing with pre-existing cells rather than by forming new cells. This finding suggests that scientists should exercise caution in using adult bone marrow cells in clinical trials designed to generate new cells in other tissues. It remains uncertain whether the fusion of blood cells with cells in other tissues can contribute to the survival or regeneration of cells in those tissues. The

researchers emphasize that their findings underscore the importance of continuing research with both embryonic and adult stem cells.

Stem cells are immature progenitor cells that have the theoretical potential to differentiate into adult cells of many types. Scientists believe that it might be possible to introduce stem cells to regenerate damaged brain, spinal cord, heart, liver and other tissues. There are many different types of adult stem cells that were each thought to generate cells from different tissues - for example, blood-forming stem cells had been thought to make only blood cells. However, studies over the past four years have observed a contribution of bone marrow cells to unrelated tissues, like heart, causing some scientists to believe that adult stem cells can mature into specialized cells of unrelated tissue types, in a process known as “transdifferentiation.” Thus, for example, hematopoietic, or blood stem cells, could give rise to mature neurons, or vice versa, if they were placed in the appropriate environment.

“The concept of transdifferentiation has been important because papers in major journals over the last few years have suggested that there is widespread potential for transdifferentiation among stem cells from a number of different tissues,” said Morrison.

“And these findings were the basis for political arguments against the use of embryonic stem cells. Some critics of this research argued that if adult stem cells really had developmental plasticity, there was no need to work on embryonic stem cells,” he said.

Other studies raised the possibility that cell fusion might account for the seeming “plasticity” observed in stem cells. Morrison, Alvarez-Buylla and their colleagues set out to develop a technique that could directly and unequivocally determine whether cell fusion actually took place *in vivo*.

Their approach consisted of installing in blood cells a kind of genetic switch, called Cre, that had the capability of turning on another “reporter” gene whose activity could be detected by a characteristic blue staining of tissues. This reporter gene is normally off in cells of different tissues. Thus, only when cells come together and fuse, does the Cre protein gain access to the switch and turn on expression of the telltale blue reporter gene.

In initial experiments *in vitro*, Alvarez-Buylla and his colleagues showed clear evidence that the system worked and it signaled when cell fusion took place.

Next, researchers in both laboratories began experiments to determine whether cell fusion occurred in live mice. In those experiments, they transplanted bone marrow cells containing the Cre protein into mice whose endogenous marrow cells had been eliminated by irradiation. Those mice had cells throughout their bodies with the reporter gene that could be switched on by Cre.

“In these mice, we consistently found small numbers of blue neurons in the brain, hepatocytes in the liver and cardiac muscle cells in the heart,” said

Morrison. Many of these blue cells had two or more nuclei, which further confirmed that they had been formed by fusion.

In additional experiments, Morrison and his colleagues inserted bone marrow cells that contained the genetic trigger under the control of DNA that caused it to be expressed only in blood cells in mice with the reporter gene. The results suggested that cell fusion was occurring between blood cells and cells in other tissues.

Alvarez-Buylla and his colleagues went a step further and performed indicator experiments that were designed to detect transdifferentiation in the cells of mice. They used a second reporter gene in the bone marrow cells that did not depend on fusion, and found no evidence that the bone marrow cells transdifferentiated into brain, heart or liver cells.

According to Morrison and Alvarez-Buylla, their findings offer caution to researchers who have already begun clinical trials in which they are inserting bone marrow cells into damaged heart tissue, in an attempt to regenerate healthy muscle.

“Our findings raise a red flag about going too fast to clinical trials based on the assumption that transdifferentiation is the mechanism by which stem cells give rise to other cell types,” said Alvarez-Buylla. “Our paper suggests that previous claims of transdifferentiation may be explained by cell fusion.” The scientists said they cannot rule out that transdifferentiation might be occurring, but that they saw no evidence of it in their experimental system.

In any case, according to Morrison, the findings emphasize the importance of using a wide range of studies to determine the properties of stem cells.

“Responsible stem cell researchers have argued all along that it was important for research to continue with both embryonic stem cells and adult stem cells,” he said. “And I think these findings further support that idea by providing evidence that the plasticity of the adult stem cells was over-estimated.

“In this paper we described a relatively simple method for looking directly for evidence of fusion. And I hope that future studies of transdifferentiation will use methods like this to determine whether the contribution of bone marrow under other conditions could also be accounted for by fusion.” said Morrison.

According to Alvarez-Buylla, the findings of the two laboratories are also significant because they might reveal a new biological mechanism.

“Although this remains quite speculative, cell fusion might be a physiologically relevant phenomenon,” he said. “While investigators have long used cell fusion as an experimental tool to explore the relative influence of one cell's cytoplasm over another's nucleus, they never suspected that fusion was occurring naturally,” said Alvarez-Buylla.

“It is possible that nature uses cell fusion to enable cells such as neurons with damaged nuclei to obtain donor nuclei from blood cells that fuse with them.

This may be a rescue mechanism for cells that are long-lived and that have no other alternative to avoid death.” Alvarez-Buylla and Morrison will now use their fusion-detection method to search for fusion in other tissues and to determine whether it does indeed play a rescue role in damaged cells.

Nonetheless, additional research will be required in animal models to determine whether there is any possible therapeutic benefit from cell fusion. Until the consequences of cell fusion between blood cells and cells in other tissues are better understood, Alvarez-Buylla and Morrison urge caution in proceeding with clinical trials that involve the injection of bone marrow cells into other tissues to promote repair.