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STEM CELLS: Controversial Marrow Cells Coming Into Their Own?

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Still a believer. Verfaillie in her Minnesota lab.

STEM CELLS

Controversial Marrow Cells Coming Into Their Own?

Despite wide skepticism, Catherine Verfaillie persevered in her research and remains optimistic that her MAP cells will one day be useful in therapy

Catherine Verfaillie of the University of Minnesota made a big splash in 2002 when she reported in *Nature* that her lab had cultivated a type of cell with some seemingly remarkable properties. Called multipotent adult progenitor (MAP) cells and derived from the stromal cells of the bone marrow, the cells seemed to be able to turn into "most, if not all" cell types, the team wrote. And when injected into a mouse embryo, they appeared to contribute to most somatic cell types, creating a so-called chimeric mouse. MAP cells were surprisingly versatile for adult cells, whose fates were generally believed to be predetermined—in fact, they looked almost as good as embryonic stem (ES) cells.

Although Verfaillie was cautious in her claims, the press seized on MAP cells as an alternative to ES cells, which are controversial because creating them involves the destruction of an embryo. Scientists, too, were eager to try them out. But in the years since, MAP cells have failed to live up to expectations. They proved hard to grow, and the talk over coffee at meetings was that no one could replicate the *Nature* work.

Last month, however, Verfaillie's cells gained fresh attention. In the January issue of the *Journal of Experimental Medicine*, a group at Minnesota and Stanford University reported that they had used MAP cells to rebuild the blood system in mice. The work has impressed one skeptic, Stanford blood stem cell researcher Irving Weissman, who collaborated on the new work. Weissman calls the result "remarkable." His skepticism, he adds, "makes me a perfect collaborator, because I insisted on very rigorous criteria for the experiments."

He emphasizes, as does Verfaillie, that these cells are clearly not as versatile as ES cells. But despite their limitations, they could prove to be useful therapeutically. "A lot of people have lost interest in MAP cells by this point," says Weissman. "What our paper will help do is get everybody to look at it again." Others agree. "I'm sure it will revive interest in MAP cells," says stem cell researcher Paul Schiller of the University of Miami, Florida.

Going to the marrow

Bone marrow basically contains two types of stem cells: those that give rise to blood, and the stromal stem cells that develop into bone, fat, muscle, and cartilage. MAP cells are derived from early precursors in this latter population.

Verfaillie's team stumbled on MAP cells more or less by accident when, in trying to grow mesenchymal stem cells (a type within the heterogeneous stromal cell population), they came up with a culture system that seemed to select for even more primitive cells (*Science*, 21 June 2002, p. 2126). The tiny cells are strictly artifacts of lab culture, requiring at least 30 population doublings before displaying some of the characteristics of ES cells.

Verfaillie was not prepared for the extreme reaction when she went public with her findings in 2002. "Certainly the perception on the part of everyone was these cells were going to do it"—that is, accomplish feats hitherto ascribed only to ES cells—says David Scadden, co-director of the Harvard Stem Cell Institute. But, he says, they turned out to be "a very fiddly cell to work with." Even scientists who obtained cells directly from Verfaillie couldn't make them perform. For example, Marcus Grompe, a liver stem cell researcher at Oregon Health & Science University in Portland, says he tried very hard but failed to get MAP cells to develop into hepatocytes in mice whose livers had been destroyed.

Verfaillie has retained her high hopes for MAP cells, although she acknowledges that, in retrospect, the original chimera result might have been wrong. "We cannot exclude currently that that is not due to fusion" rather than chimerism, she says. Fusion, in which introduced cell types merge with the locals, first came to scientists' attention shortly before the Verfaillie *Nature* paper appeared (*Science*, 15 March 2002, p. 1989) and ultimately led to disillusionment with the notion that adult cells were capable of turning into other types of cells. Now researchers believe that in

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Stem Cell Candidates Proliferate

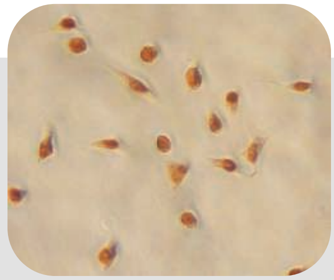
The stem cell landscape is getting crowded. Over the past few years, scientists have reported a variety of new types of stem cells, from both animal and human sources, including fetal liver, mouse testes, bone marrow, and umbilical-cord blood. One of the most recent—and widely publicized—studies, by Anthony Atala of Wake Forest University in Winston-Salem, North Carolina, described the potential of cells isolated from the amniotic fluid to differentiate into all three germ layers in vitro and into bone and brain cells in live mice (*Science*, 12 January, p. 170).

All the reports are preliminary, and none of the new cell types appears to be able to duplicate the potential of embryonic stem (ES) cells. Nonetheless, in combination, they may one day become prime players in stem cell therapy. A few examples:

• **USSCs:** A group at the University of Düsseldorf in Germany has identified a population of cells from human cord blood, which they call USSCs, or unrestricted somatic stem cells. They have "many overlapping features with MAP cells," they report, differentiating into a variety of tissues in vitro.

• **EPCs:** Douglas Losordo of Tufts University School of Medicine in Boston, Massachusetts, is currently conducting a study injecting bone-marrow–derived cells called endothelial progenitor cells (EPCs) into patients with angina in hopes of creating new blood vessels to the heart.

• **MIAMI cells:** A group at the University of Miami School of Medicine in Florida has reported a population of "pluripotent" cells from human bone marrow that they have dubbed marrow-isolated adult multilineage inducible (MIAMI) cells. MIAMI cells may complement MAP cells, they say, expressing the two stem cell markers, Nanog and Sox2, that MAP cells lack.



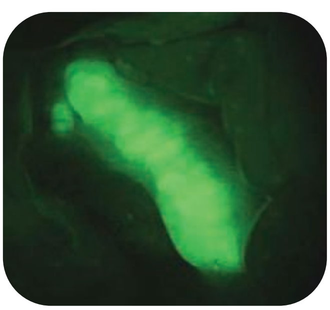
Multiple progenitors. MAP cells, such as these from rat, are just one type of stem cell under investigation.

Robert Lanza of Advanced Cell Technology Inc. in Worcester, Massachusetts, predicts that a variety of different stem cells will prove optimal for different diseases. And in some cases, stroke for example, in which blood vessels and neurons are damaged, more than one stem cell type will be used. Marcus Grompe of the Oregon Health & Science University in Portland agrees that "there's a good chance" that new stem cell therapies will rely primarily on non-ES cells. ES cells are essential research tools. But, he says, at the current state of knowledge, "I haven't met a single person who wasn't leery of them for putting into people." **–C.H.**

many—perhaps most—reported cases of so-called adult cell plasticity, the appearance of cells switching identities was in reality misleading signals from fused cells.

But Verfaillie and Weissman ruled out fusion in the mouse blood paper and say the findings are indisputable. The researchers took mouse MAP cells and expanded them with up to 80 population doublings. They then transplanted them into 28 mice whose bone marrow had been destroyed by radiation. Because MAP cells are slow to grow and thus would be unable to repopulate the blood system fast enough to save a mouse from dying of radiation, they also injected blood stem cells. In the study, reported online 15 January in the *Journal of Experimental Medicine*, the MAP cells survived and contributed to the blood systems of 21 of the 28 mice.

"Scientists must now understand that mouse MAP cells can make normal blood," proclaimed co-author Weissman. That makes them a promising treatment option because the population is readily expandable, in contrast to hematopoietic stem cells, which are difficult to expand in the lab. But first, he says, scientists would have to find a way to boost the speed at which MAP cells work, perhaps by pushing them to a more advanced stage of development before they are transplanted. And of course the study must be replicated with human MAP cells.



Blood makers. MAP cells populate mouse lymph node.

Verfaillie attributes their success in part to the development of improved culture conditions over the past half-dozen years, which are resulting in more homogenous cell populations with high levels of Oct4, the main marker they share with ES cells. Verfaillie, who has been working at both Minnesota and the University of Leuven in Belgium for the past year and is now settled at Leuven as head of its stem cell institute, has also trained a number of groups, mainly in Europe, in MAP cell cultivation.

She says some 30 papers—about two-thirds with her as a co-author—have so far been published on MAP cells, and more are in the works. Last November, for example, Felipe Prosper's group at the University of Navarra in Pamplona, Spain, published a paper in *Blood* reporting that MAP cells contribute to tissues lining the walls of veins and arteries in mice.

Verfaillie also believes the cells may hold promise for mending sick livers and other organs. Some cells, she says, may "have to commit in the dish first—the environment in vivo may not give the right signals." She says a half-dozen papers currently in preparation or under review will present evidence that undifferentiated MAP cells can differentiate to specific cell types and be of therapeutic benefit in mice. Athersys, a biotech company in Cleveland, Ohio, is licensed to produce the cells, which are patented by the University of Minnesota.

Limitations

So far, says Verfaillie, what gives her group the most trouble is trying to make nerve cells and heart cells. Nerve cells are ectodermal tissue, and "it seems easier to make mesoderm and endoderm," she says. And even though heart tissue is mesodermal, no one has had any luck coaxing MAP cells to function as heart cells either. Even in vitro, "we still can't make cardiac anything, which is strange," says Verfaillie. Nonetheless, she's more optimistic about MAP cells' ultimate versatility than is Weissman, who remains skeptical despite the recent blood success.

It's still not clear exactly how MAP cells will stack up to ES cells. ES cells are the gold standard of pluripotency—which is usually defined as the capability of generating all cell types in the adult body. There are several important markers of pluripotency, namely Oct4, Nanog, and Sox2. The ability to form benign tumors called teratomas is one of the basic tests for pluripotency, as is the generation of a chimeric mouse from injecting a single cell into a mouse blastocyst. MAP cells have Oct 4, but they lack both Nanog and Sox2. Nor can they form teratomas. "We termed them 'multipotent' because the cells definitely do not have all the features of ES cells," says Verfaillie.

Verfaillie says she regrets the hype over her findings, which she sees as "in large part politically motivated"—and by no means confined to MAP cells. With these and other types of stem cells, she says, it is too soon to predict where treatments will be found.

–CONSTANCE HOLDEN

CREDITS (TOP TO BOTTOM): CATHERINE VERFAILLIE; ANGELA PANOSKALTSIS-MORTARI