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Transparent Fish Provide Window on Blood Formation and Marrow Transplantation

Researchers have developed powerful new techniques to see in unprecedented detail how blood-forming cells develop in zebrafish. The scientists have used this system to transplant blood cells with fluorescent “tags” so they can observe how the cells restore the blood system in mutant zebrafish that do not have any red blood cells.

The techniques may be helpful in learning how bone marrow transplants reconstitute the immune systems of patients whose immune cells have been destroyed by chemotherapy during cancer treatment.

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— **Leonard I. Zon**

The researchers, led by Howard Hughes Medical Institute investigator Leonard Zon, published their studies November 9, 2003, in *Nature Immunology*. Zon and colleagues from Children's Hospital Boston collaborated on the studies with researchers from the University of California at Los Angeles.

“The zebrafish is a fantastic organism for developmental studies because the embryos are completely transparent,” said Zon. “You can watch under a microscope all the organs form within twenty-four hours in the blood system. And you can watch the heart pump blood cells through the vasculature.” Furthermore, the blood-forming systems of fish and mammals still have much in common despite the fact that the two groups diverged about 450 million years ago.

Although zebrafish are excellent models for genetic studies, there have been two major obstacles that prevented researchers from using the fish to study blood formation, said Zon. Researchers had difficulty distinguishing individual cell types and they could not transplant genetically altered cells into the blood system of the zebrafish. Zon and his colleagues have now cleared both hurdles.

The researchers used flow cytometry, a technique that rapidly separates individual cells according to their optical properties. In one set of experiments, the researchers found that they could separate distinct populations of red and white blood cells based on their light-scattering characteristics. The scientists exploited this property to study the characteristics of blood cells in mutant fish that were known to have defects in blood-cell formation.

“This surprising property of zebrafish blood cells proved to be a tremendous advantage for us because now we can use this technology to examine some of our mutant phenotypes,” said Zon. Zon and his colleagues have already used the technique to reveal that carriers of particular mutations - which were previously thought to have normal blood cells - do in fact have aberrant blood formation.

In the next series of experiments, the researchers inserted genes for either green or red fluorescent proteins into the fish, to distinguish blood cells according to their characteristic fluorescence. “We've been able to put expression of these two fluorescent proteins into distinct blood cell populations, and to use those to visualize how the transplanted cells will actually behave when they're put back into [this] easily visualized animal,” said Zon. “We believe that this ability to be highly selective about which populations of blood cells we're analyzing constitutes a tremendous advance for the field.”

Zon and his colleagues next used their fluorescent-tagging method to follow the progress of transplants of tagged blood cells into *bloodless* zebrafish mutants that lack a functioning blood-forming, or hematopoietic, system. After the cells were transplanted, the researchers could easily see precisely how the transplanted cells “rescued” the mutants by restoring their hematopoietic system.

“In our most elaborate experiments, we produced donor fish in which the white blood cells had green fluorescent protein and the red blood cells had red fluorescent protein,” said Zon. “Thus, we could follow the course of each of these types of cells as they reconstituted the hematopoietic system of the mutant fish. We found that these mutant animals, which normally died in only days, lived for many months with green fluorescent blood cells.

“We've been able to do a marrow transplant on fish,” said Zon. “We find it particularly exciting that we can now watch how these cells home in on the blood-forming organs immediately after the transplant.” The ability to track blood-forming cells and to transplant them, said Zon, will enable the researchers to analyze in detail the cellular defects in mutant fish and to

manipulate the mutants to determine which genes are malfunctioning. Such studies will have implications for both basic understanding of blood formation and for the machinery that drives immune-system reconstitution in transplantation, said Zon.

“It’s possible using our ability to distinguish and manipulate stem cells to gain a better understanding of the factors that regulate stem cell number and their ability to differentiate into a particular type of tissue,” said Zon. “Our hope is that by using the techniques we’ve developed, we can learn how to enhance stem cell production for human transplantation.

“These findings will also enable better understanding of how marrow transplantation reconstitutes the hematopoietic system in cancer patients whose blood and immune systems were ablated by high-dose chemotherapy,” said Zon. “Currently, our understanding of where transplanted cells go, how they home to the blood-forming units, and how they differentiate and proliferate is a complete black box. In the clinic, we transplant marrow stem cells, and they go off somewhere into circulation and set up home, and we only know they’ve been grafted because the patient’s immune system begins to respond.”