
RIGHT

In 2002, Juan Carlos Zúñiga-Pflücker's lab changed immunology research by growing T cells in a Petri dish. Four years later, we're seeing the products of that change

TURNS

Science is discovery as storytelling. But the story unfolds along a nonlinear path. And, eventually, it veers in unexpected directions. "That's where being a good scientist comes in," says Dr. Juan Carlos Zúñiga-Pflücker. "You try to keep making correct turns." Zúñiga-Pflücker, senior scientist at Sunnybrook Research Institute and Canada Research Chair in Developmental Immunology, and Maria Ciofani, a PhD student in his lab, have a knack for right turns. The result is a string of discoveries on T cell development, and charting of new territory in developmental immunology.

T cells are fundamental to immunity; they identify and kill pathogens, including viruses and cancer cells. Understanding the genes and molecules that generate T cells could improve on existing—but largely experimental—types of cancer immunotherapy like vaccine therapies and adoptive T cell transfer (growing large numbers of T cells for injection, shown to eliminate some late-stage cancers). It could also unleash the enormous potential of T cell-targeted pharmaceutical cures for cancer and AIDS.

Sometimes, the correct turn is a 180. In 2002, Ciofani's PhD work wasn't going anywhere. She was trying to define genes and molecules that control T cell development in vivo—because T cell progenitors mature into T cells only inside the thymus. (The thymus is a small organ in the upper chest, to which T cell progenitors migrate from bone marrow.) But that year, Tom Schmitt and Zúñiga-Pflücker induced T cell development in a Petri dish by growing preclinical stem cells, with Delta-like-1 molecules, on supporting stroma. For the first time, scientists could view in molecular detail a process that had stymied them for 40 years. "It saved my degree," Ciofani says.

She and Zúñiga-Pflücker closed the book on her experiments and started down a new path.

Growing large numbers of previously hard-to-find early T cells in vitro, and with a new view into the signalling role of the Delta-like-1 molecule in differentiation (the crucial point when stem cells are directed to choose a lineage and become a specific cell type), Ciofani made a striking discovery about the receptor for Delta-like-1, known as Notch. Notch wasn't only instructing stem cells to become T cells, it was sustaining them after differentiation via another molecule inside the cells, called Akt, and ordering them to take up the nutrients required for their survival. In a fashion described by Zúñiga-Pflücker as typical of her—perfectly laid-out experiments and a rare ability to place them in a larger scientific context—Ciofani took a sharp turn from cell signalling to metabolism, literally looking into the microscope to recognize that removal of Delta-like-1 atrophied the T cells.

Beyond providing a critical piece in the T cell development puzzle, the discovery had clinical relevance for T cell acute leukemia (TALL), in which T cells turn cancerous. Notch is mutated in 50% of TALL patients, so pinpointing how and at what stage it promotes T cell growth opens a promising avenue for investigation into controlling its cell-sustaining effect during TALL. The discovery was featured on the cover of *Nature Immunology* in 2005.

In 2006, Zúñiga-Pflücker and Ciofani extended this finding. By selectively withdrawing Notch signals in vitro at various stages of T cell development, they determined at precisely what point the two main types of T cells—alpha-beta and gamma-delta, each with particular roles in protecting us

MARIA CIOFANI

THE COLOUR OF FLOW
NEW TECHNOLOGY LIGHTS UP THE NATURE OF BIOLOGY

For the *Nature Immunology* paper on the role of Notch in sustaining T cell development, Maria Ciofani, though she could see the cells shrinking by comparing slides in the microscope, needed to quantify that change. She used Sunnybrook Research Institute's state-of-the-art Centre for Cytometry and Scanning Microscopy to produce the requisite numbers on decreasing cell size and volume. The centre is essential to the work done at SRI by several molecular and cellular biologists. One important new piece of equipment it houses is the LSRII by BD Biosciences, funded primarily by the Canada Foundation for Innovation and Ontario Innovation Trust.

The LSRII is a $450,000 flow cytometer used to analyze and characterize cells and their environment after they've been sorted. It beams four independent laser lines onto cells suspended in liquid and stained with dyes to illuminate their various characteristics in up to 16 colours. A standard flow cytometer produces four colours with two lasers. Immunologists trying to characterize cell phenotypes, for example, use antibodies tagged with fluorescent dyes to look at proteins that are expressed by certain genes inside and outside the cells. Advances in this technology have dramatically increased the rate of scientific discovery: cell analyses that took 45 minutes 15 years ago now take less than 10 seconds and give much more detail.

Several functions of the LSRII, the most advanced analyzer on the market, couldn't have been performed at all—even two years ago. The multiple fluorescence protein array, which allows for analysis of multiple genes at once that have been tagged with different fluorescent dyes, is an example. Genes are introduced into cells, either transiently or permanently, and tagged with red, green or yellow dye. Gisele Knowles, who runs the flow facility, says, "From the 1990s until 2004, only one dye—green—could be used for that type of analysis. Now we can use three with the LSRII only. It means we can see the effects of three different genes in a cell at once. That's huge."

As biologists shift their focus from identifying the presence or absence of individual genes in disease to looking at how various genes interact at specific points in time—genomics—the LSRII is enabling SRI to stay at the forefront of genetics research.

Having the equipment is essential, but using it effectively can take cellular research to another level. Knowles, the first in Canada to get a sorter and with 22 years of experience to draw on, provides the direction to make that happen. "I'll probably never work with another flow technician of Gisele's calibre," says Renée de Pooter, a frequent user. "She understands the physics of the machines and the biology of the cells you're trying to sort, and that's extremely rare. She's amazing." JO

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from disease—bifurcate from a common T cell progenitor. Only gamma-delta cells will continue developing into mature T cells, despite the absence of Notch function beyond the early progenitor stage of development. While furthering the understanding of Notch, the finding also clarified how to generate the two types of T cells in the lab, thereby facilitating translational research on gamma-delta cells in adoptive T cell transfer therapy. Gamma-delta cells have robust, specific antitumour properties and are much less prone to autoimmunity, a common problem with adoptive transfer.

While Ciofani was mapping preclinical T cell development, Dr. Ross La Motte-Mohs, a postdoctoral fellow in Zúñiga-Pflücker's lab, added weight to her work by replicating their in vitro system with human stem cells drawn from cord blood. Zúñiga-Pflücker says, "It's critical to have the fundamental understanding of genes, molecules and cells, but it becomes a lot more important when you have the human correlate in place and shown playing a role." Published in *Blood*, the results translated to the human system and, says Zúñiga-Pflücker, "on some levels, provided a more complete sense of T cell development than in the preclinical experiments." La Motte-Mohs and Zúñiga-Pflücker are now partnering with researchers and industry in the Advanced Regenerative Tissue Engineering Centre (see page 39) to explore the feasibility of constructing an artificial thymus for T cell transplantation.

In autumn 2006, Zúñiga-Pflücker confronted the reality that Ciofani will finish her PhD and leave the lab early in 2007. "It's the bittersweet aspect of this work," he says. Once students have developed their experience, worked through failed experiments and multiple forks in the road—just when the science is, one hopes, showing results—they leave. "All you can hope is that they go out and get what they're after," he says.

What is Ciofani after? She's moving to Manhattan to pursue T cell development as a postdoctoral fellow at Memorial Sloan-Kettering Cancer Center. "I want to know," she says. "I think that's probably a little part of every scientist—needing to know the answer. And this is a bit of egotism, but I want to know it first." It is that drive and curiosity that move the story of science forward. JO

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