New tool reveals secrets of migrating cells

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Biologists have a new tool to track and videotape cells moving about inside living tissue. Called two-photon laser-scanning microscopy, it has revealed, for example, the dramatic difference between the random wanderings of immature T cells and the goal-oriented, beeline movement of activated T cells.

"This is the first time anybody has quantitated four-dimensional data - spatial and time data - to get a picture of long-range cell migrations through tissue," said immunologist Ellen Robey, professor of immunology at the University of California, Berkeley. "The ability to directly visualize cells in living tissues has changed the way immunologists think about how cells explore their environment, how they signal to each other, and how they migrate."

Robey and post-doctoral colleague Colleen Witt are among a handful of researchers using two-photon imaging to obtain real-time images of cells throughout the top half-millimeter of a living organ, not just on the surface of tissue or within a slice.

"In our earlier studies (published in Science) we could see cells getting together, presumably signaling one another. In our current work, we observe cells that we believe have already gotten a signal beelining away," Robey said of her studies in the thymus, the immune system gland that weans baby T cells into activated helper, or CD4, cells and killer, or CD8, cells primed for combat with viral invaders. "We were surprised by how rapidly and directly the cells move to their final destination."

The technique could allow researchers in many fields of biology to track migrating cells, which biologists have discovered are common in many types of tissue, ranging from nerves to lymph nodes. To date, such long-range migrations have been inferred from observations of chemically fixed tissue at different stages of development.

"Two-photon imaging is going to change literally forever the way that we do biological science," said Witt, a developmental immunologist. "In the past, we'd take organs out, smush them up and basically do biochemistry in test tubes, or watch their behavior in a single layer of cells. It's an imaging revolution to be able to go into the native environment while keeping the intact organ alive and make movies of migrating cells."

With two-photon imaging, Witt and Robey identified thymus cells they dubbed beeliners moving nearly two centimeters - almost an inch - per hour, which is fast in the realm of cell movement. They think that these are cells that have received a signal committing them to be either a helper T cell - which aids other immune cells in fighting infections - or a killer T cell that seeks and destroys cells infected with virus.

On the other hand, uncommitted or immature T cells, what they call meanderers, wander slowly and apparently randomly around the outer layer, or cortex, of the thymus, perhaps in search of that life-altering signal.

Robey hopes to use two-photon imaging to investigate the signals responsible for changing these meanderers into purposeful beeliners that immediately leave the cortex for the interior medulla of the thymus.

"We're now at the point with this technology that we can begin to look at the movement of signaling molecules within the cells," she said.

Robey, with another colleague, Philippe Bousso, last year published a

review in the journal Immunity describing the contributions two-photo imaging has made to the field. Robey and Witt publish their current study in the May 3 issue of the Public Library Of Science-Biology.

Two-photon imaging is a variation on the standard technique of labeling cells with fluorescent dye and then hitting them with a laser that makes the dye glow and the cells light up. A certain energy or color of laser light is needed to make the dye, in this case green fluorescent protein, glow. But high-frequency, short-wavelength visible light, like green, doesn't penetrate tissue as deeply as longer, redder wavelengths.

The idea behind two-photon imaging is that if you hit a dye molecule in a short period of time with two photons of light, each photon half the energy needed to excite it, the dye can absorb them together and then fluoresce. The less energetic, long-wavelength photons will go deeper into the tissue, cause less damage and scatter less, Robey said, essentially illuminating slices through the tissue that can be sharply imaged and stacked to produce a 3-D image of the cells in real time. The system they use employs an infrared laser emitting short intense pulses of 920 nanometer-wavelength light.

In the thymus, it's possible to view cells 400 microns inside the cortex, which is about 4/10 of a millimeter or more than a hundredth of an inch deep. In the current study, Witt limited her viewing to about 200 microns, though she says in some tissues less dense than the thymus, light could penetrate nearly a millimeter - deep enough to probe cell activity in most tissues.

Witt pointed out that obtaining a movie of cell movement is just the beginning. The human eye and brain can't pick out patterns of movement easily, so statistical techniques are needed to identify cells with different patterns of movement.

As an immunologist, Robey focuses on the lives of T cells produced in the thymus and distributed via the bloodstream to the lymph nodes, whence they move into the body's tissues. Her first use of two-photon imaging three years ago surprised her and many immunologists because it showed that thymocytes or immature T cells were highly mobile, traveling thousands of microns in an hour as they explore the thymus.

The new experiments, conducted primarily by Witt, suggest that this exploration probably is a search for a signal that will decide the cell's fate. In their experiments on one lobe of the mouse thymus, in fact, Witt and her colleagues saw cells, possibly those that have made a decision on their fate, halt their wanderings and make a beeline out of the cortex to the medulla, something immature T cells can't do.

The scenario they've reconstructed from the video and mathematical analysis starts with immature T cells moving out of the center of the thymus, the medulla, to the very outside edge of the cortex, where they proliferate and fill up the cortex. At this point, Witt said, they undergo the first of two tests to see if their surface receptors (called T cell receptors) work properly.

Once they pass that test, they start wandering around in the cortex looking for the second test, which is to bind precisely to a protein called the major histocompatibility complex (MHC). These cells, the researchers think, are the meanderers. Only about one percent of thymocytes pass both tests, but Witt and Robey think that those that do are the ones they see beelining out of the cortex into the medulla to begin their two-week education to distinguish "self" from "non-self" invader.

"To pass into the medulla they have to pass a screening test called positive selection," Witt said. "Once they do, the cells move very directly at a very fast speed inward toward the medulla, adopting a polarized

shape characteristic of migrating cells."

While Witt and Robey continue their two-photon imaging studies of thymus cells and lymph cells, Witt is trying to encourage the technology's use in biology generally.

"Immunology is just one example of a subdiscipline of biology that stands to benefit enormously from our new ability to see in four-dimensions - in 3-D in real time. It opens an entirely different universe to us," Witt said.

Coauthors with Witt and Robey are Arup Chakraborty, UC Berkeley professor of chemistry; Subhadip Raychaudhuri, formerly of UC Berkeley's chemistry department but now with the Department of Biomedical Engineering at UC Davis; and Brian Schaefer of the Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, Md.

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