Michael Sixt: Love the way they move

Sixt studies the mechanisms of leukocyte locomotion.

A cell's job description is reflected in both its physical form and its behavior. For example, fibroblasts and epithelial cells need to stick tightly to each other to hold tissues together. So they tend to spread out, hold tightly to surfaces, and move about only slowly. Leukocytes, on the other hand, need to move about a lot and cover large distances as they patrol the body for signs of infection, so they're extremely nimble and their shape more malleable.

Ever since his thesis work in medical school, Michael Sixt has been fascinated with leukocytes' lively behavior (1). His lab is investigating the molecular (2, 3) and mechanical (4, 5) paradigms at work in leukocyte locomotion, exploring how the strategies leukocytes use to get about differ from those of other cells. We called him at his lab in at the Institute for Science and Technology (IST) Austria to get the inside scoop on these slippery cells.

AMPHIBIOPHILE

What was your first exposure to biology?

I grew up in Bavaria, near a small city called Weiden, close to the Czech border. The village I grew up in had only 15 inhabitants, including my parents, who were physicians working in the city, and two neighboring farms. I went to school in that city, but I spent much of my early childhood in the forest.

> "I realized that studying cell behavior was really my thing."

When I was 15 or 16, I was very interested in bird watching and also in amphibians. I actually had an amphibian rescue program, collecting toads from the roads at night so they wouldn't get run over. I can't remember how I came to do this—I think it was something my sister had started doing that I then took over—but I also started to do some science on these toads, analyzing their sex ratio and so on.

Did that make you want to be a scientist?

Yes. At first I thought I'd study biology, but in Germany at that time everyone had to do a few years of either military or civil service after completing high school. So for one and a half years I did my civil service on a North Sea island, working on an environmental protection project. I was counting birds, making maps of breeding birds, and so on. I worked with some professional ornithologists, and I just thought that the projects were not very thrilling or interesting. That is why I ended up studying medicine instead of biology.

As part of getting an MD in Germany, you have to do a small thesis, somewhat similar to a master's degree. I did mine in a basic science lab with Lydia Sorokin. It took a few years before I realized that research can give some really interesting results, because of course things go very slowly early on and it is very difficult to get those first results. But after a time I got really interested.

The decisive moment for me, when I really knew that I wanted to do science, was during my clinical residency in the dermatology department at Erlangen led by Gerold Schuler. There was a strong immunological research program, and in addition to my clinical work I started to make movies of cells, especially of dendritic cells moving around in culture. I realized that studying cell behavior was really my thing, maybe more than seeing patients. By this time Lydia Sorokin had moved to Sweden, and my wife wanted to go to Copenhagen for her postdoc in evolutionary biology, so I joined Lydia's lab again for a postdoc.

LOOK AT THEM GO

You were most interested in cell motility…

The paradigm of cell motility on two-dimensional surfaces is that F-actin filaments polymerizing at the front of the cell push out the membrane and at the same time push themselves back into the cell. If this retrograde flux of the filaments is coupled to the environment, for example through integrin-based adhesions, then this pulls the cell forward.

PHOTO COURTESY OF IST AUSTRIA

Michael Sixt

Fibroblasts and epithelial cells are very adhesive. They need a lot of contractile force to pull themselves forward and detach themselves at the back, so they're relatively slow. If you look at professionally migrating cells—leukocytes—crawling on a two-dimensional substrate, they too use this principle of adhering and generating traction by actomyosin contraction. But once you put them in a 3D environment, they get much more flexible. My lab has found they can actually move in the absence of integrin-mediated adhesions, and, because they don't have to assemble and disassemble these attachments, they can move much, much faster.

Why did you choose to study this specifically in dendritic cells?

Other leukocytes must first extravasate across the endothelium, from the blood into the tissue, and this step is dependent upon integrins. My lab is actually interested in the locomotion of all kinds of leukocytes, but during my residency I started to work on dendritic cells and found them a very useful model system because they never have to do this extravasation step. They start out sitting in the tissue and then migrate through the tissue into lymph vessels, toward the lymph node.

Text and Interview by Caitlin Sedwick
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When I started my group at the Max Planck Institute in Munich, I was hosted by Reinhard Fässler's Department. He's a big guy in the integrin genetics field, and he had this huge collection of mouse mutants that he was very generous with. Using conditional knockouts of the β1, β2, αV, and β7 chains, we showed that dendritic cells don't need integrins to travel from tissues to lymph nodes.

MYSTERY OF MOTION

How do these cells achieve forward motion if they don't use adhesions?

One thing we looked at in the beginning was the contribution of actomyosin contraction to the cells' motility. And that turned out to be the main driver at the trailing edge of the cell; it acts as a kind of contractile cage around the nucleus. If you look at a three-dimensional interstitium, such as a collagen matrix, it's full of pores. And when the cell migrates, the nucleus is the stiffest part of the cell, so it frequently gets stuck trying to fit through the pores. In order to overcome this, the actomyosin at the back of the cell contracts and squeezes the nucleus through the constriction.

> "We have a favorite hypothesis about this."

But the main driving force of locomotion is, of course, actin polymerization. To study this, we developed methods to visualize actin flow in cells, and we also produced more reductionist setups, where we don't look at the cells migrating in vivo or in three-dimensional collagen gels but instead place them between two surfaces. We can use surface patterning to create either adhesive or nonadhesive substrates and then use total internal reflection microscopy to see how the cytoskeleton behaves on different surfaces.

What we found there is that the cells can move on both sticky and slippery substrates. When they're on a sticky surface they exhibit adhesion-dependent motility, but when they're on a slick one they enhance their actin flow to compensate for slippage. We still don't understand how they achieve traction in this scenario: are there alternative receptors that couple the retrograde actin flow to the extracellular environment? Or are they utilizing the topography of the environment? We have a favorite hypothesis about this: if they're in an irregular three-dimensional environment, perhaps they can push an arm into this environment, inflate it, and then move forward.

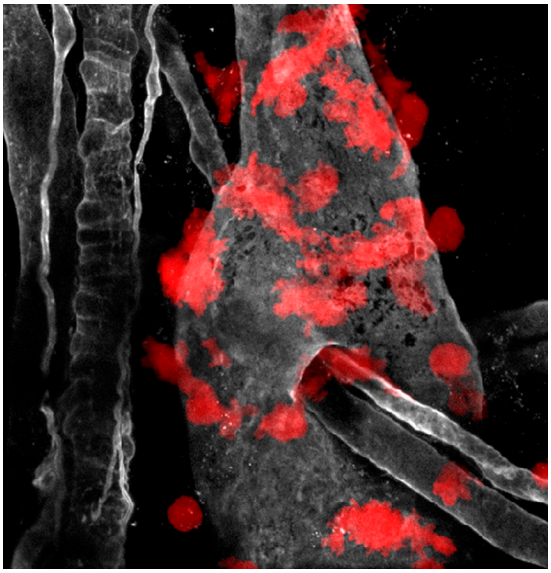

IMAGE COURTESY OF HOLGER PFLICKE

Dendritic cells (red) about to move into a lymphatic vessel (white).

You've also looked at how migration is directed by chemokines…

We work on a particular chemokine receptor, CCR7, and its two ligands, CCL21 and CCL19. It sounds like a very specialized question, but it's a useful model system because it lacks all the redundancy and inflammatory properties of other chemokines. Also, CCL21 has very strong matrix-binding properties, whereas CCL19 is probably mostly soluble in tissues, so it's interesting as a paradigm for how guidance cues act on cells, especially immune cells, in vivo.

Dendritic cells only use CCR7 as their guidance cue receptor on the way to the lymph node. We know very little about what role CCL19 plays because it is difficult to detect concentration gradients of soluble cues. What we know so far about CCL21, with its strong matrix binding, is that it is present in a spatial gradient within the skin. We can reconstitute this behavior in vitro, and we've shown that this spatial gradient is used by dendritic cells as a guidance system to crawl from the interstitium into lymphatic vessels.

I would like to find out how cells interpret this gradient. Are they using spatial sensing, or is there also a temporal component, like in bacteria? This, together with our work on actin dynamics and force transduction, is occupying a lot of my lab right now. We're also interested in the polarity modules that segregate the front and back of the cell and how these are affected by cell shape.

These questions are taking our work in a very multidisciplinary direction. About three years ago I joined the IST, which is a new and very interdisciplinary institute. It's a nice environment for us.

PHOTO COURTESY OF MICHAEL SIXT

Sixt's children agree that watching cells move is pretty fun.

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