Gillian Griffiths: How T cells get on target

Griffiths studies how cytotoxic T cells deliver their lethal payloads.

Cytotoxic T cells patrol body tissues in search of cells that are infected with pathogens and immediately kill any they find. For the T cell, the killing process involves dramatic rearrangements of its cytoskeleton and of secretory granules containing cytotoxic proteins. Gillian Griffiths blends the fields of immunology and cell biology to investigate how T cells land their killing blow.

Building on a strong pedigree in immunology, Griffiths first studied the contents of cytotoxic T cell granules (1, 2). This naturally dovetailed into the question of how T cells deliver these granules to target cells. Her careful observations of the killing event have led to several revelations concerning the mechanisms behind this phenomenon (3–5). We called her at her lab at the Cambridge Institute for Medical Research in the UK to learn more.

PHOTO COURTESY OF JIM KAUFMAN

Gillian Griffiths

THE PROPER APPROACH

What got you interested in immunology?

When I was in school I really wanted to become an ecologist, but that didn't quite happen, because I went to University College in the center of London. Because everybody there told me how wonderful the immunology course was, I signed up.

At that time the immunology class was run by Martin Raff and Avrion Mitchison. They were so clearly excited about the subject. They gave the sense of how much there was to discover and how much fun it was to discover it. It's interesting because there are several cell biologists and immunologists who emerged from that department. So I was lucky that I happened to find the right people at the right time.

Speaking of good timing, your graduate advisor received the Nobel Prize while you were there…

I knew I wanted to do a PhD, and I knew I wanted to continue in immunology. When someone suggested I approach César Milstein at the Laboratory of Molecular Biology in Cambridge, I'm embarrassed to say I'd never heard of him. During the period that I was at the LMB, someone at the institute seemed to win a Nobel Prize more or less every year. So you kind of got used to the fact that every October there'd be champagne in the cafeteria, and each year everyone said, "Oh, it'll be César next, for his work on monoclonal antibodies." So it was really good to be there when he won his prize.

The great thing about César was he ran a small lab and he thought through the projects that he had his students work on very carefully. When I first got to his lab, I was very naïve. I didn't know why I was given the project I was working on. The LMB at the time was full of very ambitious American postdocs who would always come up to you and ask, "What are you working on?"

When they asked me this and I replied that I was sequencing B-cell receptor V genes, they'd turn around and say, "But everyone's doing that. Why are you doing that?" And I scratched my head and went back to César and asked him, and he smiled and said, "Ah, but they're not doing it properly."

> "What hadn't struck me at the time was what an unusual mechanism this is."

Lots of other labs were sequencing the amino acid sequences of V genes, but what César had me do was to sequence the messenger RNA. This allowed us to get a much longer stretch of the nucleic acid sequence, so we could identify the somatic mutations taking place in these genes.

A NEW AIM

Why did you not keep working on somatic recombination after that?

I thought that I'd be a better scientist if I did something really quite different within the field of immunology rather than continue working in the somatic mutation field. And because the LMB was full of Americans, the advice that I got there was, "Well, now you've got your PhD, you've got to get your BTA," which stood for "been to America." [Laughs]

Irving Weissman's lab at the time was looking at lymphocyte homing. I thought that was really exciting and intriguing, so I went off to work with Irv. But T lymphocyte homing turned out to be really complicated to study at that time, and I eventually turned my attention to a protein Irv's lab had identified, called granzyme A. The suggestion was that I use this as a marker to see where you could find activated T cells in, for example, arthritic patient samples. It was a very different kind of work, and I realized that it perhaps wasn't what I wanted to do in the longer term. What fascinated me, though, as I learned more about these different proteins, was that they are packaged into specialized granules that are secreted from cytotoxic T cells and are responsible for their killing activity. I thought that was a very interesting idea.

I really wanted to understand how lytic proteins were packaged into these granules. I was inspired by the work of Stuart Kornfeld and others, who used human genetic diseases to understand how lysosomal proteins were packaged. I found this incredibly elegant, and one of the first

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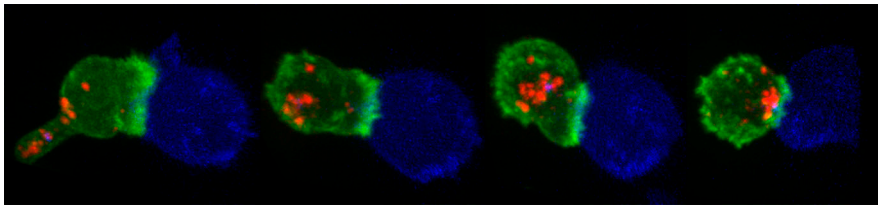

IMAGE COURTESY OF ALEX RITTER

Single frames from a movie showing the polarization of a T cell's lytic granules (red) and actin (green) toward its target cell (blue).

experiments I did when I set up my own lab at the Basel Institute for Immunology was to use cytotoxic T cells from patients with Inclusion-cell disease to show that the granzymes used the same mannose-6-phosphate receptor pathway to reach the granules and that this was important for killing by T cells. This made me realize just how much cell biology could be learned from studying genetic diseases, and this is something that I continue to do.

What was known at that time about how killing is achieved by cytotoxic T cells?

It was known from the work of Avi Kupfer and Gideon Berke and others that the microtubule-organizing center (MTOC) polarizes towards the contact site between a T cell and its target cell, at the immunological synapse. So it was thought that the secretory granules move down the microtubules towards the MTOC, which is polarized towards the target cell.

What wasn't clear were the last steps of killing. We wondered if there are actually short microtubules emanating out from the MTOC that take granules that last step to the immunological synapse or if granules reach the cell surface in some other way. And so it was a real revelation when Jane Stinchcombe's electron microscopy showed that, in fact, the centrosome—which resides at the center of the MTOC—comes all the way up to the plasma membrane and docks at the center of the immunological synapse, where the T cell receptor (TCR) clusters. What hadn't struck me at the time was what an unusual mechanism this is, because in most cell types secretory granules move away from the MTOC to get to the plus end of microtubules, which are anchored at the plasma membrane. And in fact, cytotoxic T cells have reversed this. It's very clever, actually, because it allows them to direct all their secretion to a single point. This is exactly what they need to do, because they need to kill that target cell very precisely, without killing other nearby cells.

"When there's a weak signal, secretory granules don't polarize towards the centrosome."

ZEROING IN

How are T cell MTOC reorientation and T cell killing coordinated?

One of the great things about working at the interface between cell biology and immunology is that there are many immunological phenomena to look at. For example, it's known that the killing of target cells by cytotoxic T cells depends on the strength of the signal that the T cell receives through its TCR. When there's a weak signal, killing doesn't occur. This seemed like something that we absolutely had to try to understand: how does the immunology translate into cell biology?

What we've found, to our surprise, is that the centrosome polarizes toward the target cell in response to both strong and weak signals. The difference is that, when there's a weak signal, secretory granules don't polarize towards the centrosome. If you look at migrating T cells, you see granules dispersed throughout the cell, so there's some signal that triggers the granules' recruitment to the centrosome. That's something we're slowly beginning to understand, but we're not there yet.

Your immunology definitely has a strong cell biology tilt to it…

Absolutely, and we are fascinated by the structure that the centrosome makes when it docks at the immunological synapse: it looks to us like a frustrated cilium. What we've been having a great deal of fun trying to understand is just how accurate that statement is and how much like a cilium the immunological synapse is.

Of course, the technology for imaging live cells has been improving all the time, and we now have the ability to look at these cells in real time using markers. You can see all these structures in live cells, and it has been a lot of fun to do that. It takes a lot of time, but I'm fortunate to have students and postdocs who really love doing this, as do I.

And outside the lab?

My life revolves around my family. My husband, Jim Kaufman, who I met in the Basel Institute, is also an immunologist. We have two children, who are the center of my universe.

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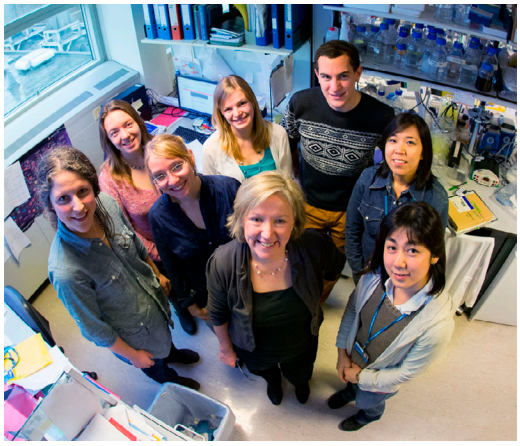

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Griffiths and (most of) her group.