Digital video vignettes of the immune system in action are opening scientists' eyes.

Lymphocytes, camera, action!

BY MARC WORTMAN

illustration by Jonathon Rosen

"You can't understand complex, changing natural phenomena with just one snapshot.... With video imaging,

In a laboratory at Stanford University School of Medicine, graduate students and postdocs spend a lot of time watching movies. Their mentor, HHMI investigator Mark M. Davis, doesn't mind a bit. In fact, he encourages them, and proudly shows off the product of a protégé's doctoral thesis, which he unofficially titles, "Immune System: The Movie." The student composed digital video recordings of immune cells going about their machine-like business—not unlike Hollywood's "Terminator"—of seeking out, recognizing, and destroying (or stimulating) other cells.

Davis calls up a video on his monitor showing the immune system in action. He watches three cells clump together, much like a basketball and two softballs lined up in a row. The largest of the three is a cancerous lymphoma cell. The two smaller cells—one blue, the other red—are components of the immune system scanning the unhealthy cell and communicating with one another about what they are "seeing."

The time-lapse images follow the two immune cells as their colors swiftly intensify and change to green. This color change is a laboratory-generated display of the internal biochemical changes the immune cells undergo when they recognize the lymphoma cell and signal to other nearby immune cells to mobilize against it. Their murderous business is swift and relentless. Nearly a dozen other cells charge in like a vengeful mob. With their colors intensifying and changing much like the first two cells, they cluster around the lymphoma cell and prepare to kill it.

Davis's team has recorded numerous videos of fluorescently tagged proteins on the surface of the immune system's T lymphocytes—the specialized white blood cells that move through the body with the flow of blood until they bump up against foreign or diseased cells. If the T cell's surface proteins link up with a sufficient number of counterpart proteins on the unhealthy cell, the T cell recognizes it as an enemy. At that point, the immune system swings into attack mode against the invader.

Only with recent advances in visual imaging systems have Davis and other investigators been able to generate these types of live-action videos. Their productions are changing the way scientists think about the immune system.

The imaging systems couple ultra-high–resolution microscopes with lasers (which send out pulses of light that illuminate fluorescently labeled protein probes, even deep within the intact tissues of living animals). These systems, known as multiphoton microscopes, include special video camcorders that produce layers of images at different microscopic depths as well as post-production software that recomposes the images into three-dimensional videos. Thus equipped, scientists like Davis can watch how the immune system works at the nuts-and-bolts level and observe what happens when it goes awry.

"You can't understand complex, changing natural phenomena with just one snapshot," says Davis. "We want to see where the molecules are, what they are doing, and how an organism responds to a threat. With video imaging, we can look at the gears turning and what cells do and how they do it."

MORE THAN ENTERTAINMENT

Microscopic observation of living cells on a slide (in vitro) or in a living organism, which goes by the general name of "intravital microscopy" (IVM), is not new. It was pioneered by German physiologist Rudolph Wagner in 1839. But the present sophistication of the process and the level of resolution now possible are indeed new, and filled with promise. When Davis and others began to generate videos in

FROM LEFT TO RIGHT:
Mark M. Davis, Dan R. Littman, Philippa Marrack

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Davis: David Powers Littman: Chris Denney Marrack: Louie Psihoyos / Science Faction Images

the late 1990s, however, some in the field questioned their value. They were seen as a fancy way of showing what scientists already knew through static images.

Coming up against such attitudes, Davis had trouble finding a journal willing to publish his early papers. Editors feared they would be opening the doors to ridicule about the MTV generation taking over scientific research. "The convention was that videos were more about entertainment than information," he says. "It was almost impossible to persuade people that video can have much more information than a still image." Soon, though, as new knowledge began emerging from video microscopy, the same editors were clamoring for him to submit more video-based papers.

Now, those dramatic images have shown that the immune system is far more dynamic and actively choreographed than previous static-image studies had led scientists to believe. Davis and others are zooming in on that activity in molecular detail. Until moving images showed them otherwise, most biologists thought that the signaling process leading to an immune response required hours or even days of continuous communication between T lymphocytes and antigen-presenting cells (the cells that engulf cells infected with viruses and, through communication with T cells, initiate the process that will kill the virus).

Video microscopy revealed, instead, that these two fundamental immune-system components engage in a day-long minuet beginning with multiple short contacts. Each lasts only a few minutes, yet these fleeting encounters prove sufficient to activate the T cells. "Few people anticipated the enormous rapidity with which cells move," says Ulrich H. von Andrian, an immunologist at the CBR Institute for Biomedical Research, an affiliate of Harvard Medical School, and a leader in the use of video microscopy.

Many unstable cellular structures collapse when they are prepared for static observation. As a result, says von Andrian, static studies may have given scientists a false conception of living immune system mechanics. Studying the immune system in its natural state, he says, "provides an essential reality check for determining which phenomena are different in living animals and not faithfully reproducible" statically. Davis agrees: "It's like seeing an animal in its natural environment, rather than in a zoo. It's really important to see where they are and how they behave in different stages of their lives in their native habitat."

THE SECRET LIFE OF THE LYMPHOCYTE

Nearly all real-time knowledge of the immune system comes from studying T cells circulating in the blood. Yet, while a T cell typically spends only about 30 minutes in the bloodstream, it might spend hours or even days migrating through other organs, querying cells for antigens. Because "there is no evidence out there for what goes on inside an organ," says Dan R. Littman, an HHMI investigator at the New York University (NYU) Medical Center, only a small fraction of the life of the lymphocyte has ever been observed. He and others have begun to open up that hidden life.

In his laboratory, Littman, in collaboration with Michael Dustin at the Skirball Institute of NYU, uses IVM in mice to observe the living immune system within organs that are accessible by surgical procedures. He started with the liver, where natural killer T (NKT) cells, the immune system's sentinels against virus-infected cells, have long been known to concentrate. Scientists had observed NKT cells in the bloodstream, but little was known about how they functioned within the complex stew of nutrients, toxins, lipids, and other chemicals trapped in the labyrinth of microscopic

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vesicles that pervade the liver. By opening a flap in the membrane covering the organ, the researchers could deploy IVM to observe and record fluorescently labeled NKT cells going about their business.

Nothing in previous studies of NKT cells prepared the scientists for what they saw. Like other lymphocytes, NKT cells get pushed along by the blood's flow through the circulatory system. But inside the liver, their behavior is entirely different. The video images showed little self-propelled machines that crawled, amoeba-like, through the organ's tiny blood vessels. They moved swiftly yet seemingly at random, passing one another, changing direction, and even traveling against the flow of blood. Such apparently directionless, self-generated surveillance behavior—which continued until the NKT cells detected damage or infection and stopped in the vicinity of the problem to launch an immune response—had never before been observed.

NKT cells are believed to play an important role in inflammation and may be involved in triggering chronic hepatitis. Now, says Littman, armed with knowledge about their normal movement in the liver, "We need to get at the mechanistic aspects of the NKT cells' surveillance behavior. Can we manipulate it in disease systems?" Developing ways to regulate that behavior could potentially lead to treatments that reduce the inflammatory response in hepatitis and other liver diseases.

Courtesy of the University of California, San Francisco

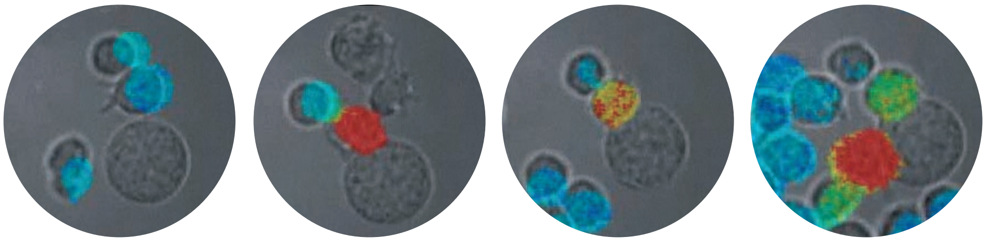
Attack of the hungry hookworm

Watching how the immune system responds throughout the body to a localized threat has provided new insights into autoimmune disorders, asthma, and allergies. Richard M. Locksley, an HHMI investigator at the University of California, San Francisco, has engineered a mouse with fluorescent probes in its immune-signaling system that light up when mucosal barriers, such as the intestinal lining or lung, come under attack. He introduced hookworms into the mouse's gut and then sliced and analyzed tissue from the entire mouse to find where the immune cells that signal such an attack, called effector cells, glowed. "This allowed us to find where every effector cell in the body ended up," Locksley says. As expected, certain known types of effector cells lit up in the intestinal lining where the hookworms bit. He was surprised, however, to find effector cells widely distributed, even in areas such as the lungs where the worms had not been. Watching these cells appear in such large numbers in the lungs in response to intestinal worms led Locksley to believe he had identified a response that overlaps with the lung's response to airborne irritants in asthma and other allergic disorders. He has made the mouse model freely available to the scientific community, encouraging others to use it to test new therapies for hookworms or other parasites, and to monitor effector cell activation and movement into unexpected places, such as the lungs and skin. "It's early days," he says, "but I'm convinced we're on the right track to show how these cells might contribute to chronic diseases like asthma. Eventually, manipulating the distribution and survival of these potent effector cells may provide new pathways for treating these diseases."

reproducible" statically.

ULRICH H. VON ANDRIAN

Still frames from a video of T cells interacting with GFP-labeled (green) antigen-presenting cells. Color overlaid on the cells highlights the intracellular calcium concentration of the T cells: Blue indicates low concentration; red is high. ***To watch the video, visit http://cmgm.stanford.edu/hhmi/mdavis/***



Courtesy of Davis lab

NEW PREDICTIVE POWER

The surface proteins, or ligands, on an invading cell must dock in a key-in-the-lock fashion with the T cell's own surface receptors for the T cell to launch an immune response. But Davis, who gained wide attention two decades ago for identifying and cloning T-cell receptor genes for the first time, observed that the binding of just one or two receptor-ligand pairs was not enough to signal the mobilization of an immune response. Because the videos that Davis's laboratory produces are so exquisitely precise that a viewer can literally count how many ligands a T cell must "see" before it reacts, he and his colleagues were able to observe that it takes at least 3, and typically around 10 ligands, for the immune system to spring into action.

"In the long term, [quantifying such interactions] is the way to determine that a certain input creates certain consequences for a cell," says Davis. "And you can only do this by imaging. That's how you get to the predictive power that has not been a part of cell biology before." As director of Stanford's Institute on Immunity, Transplantation, and Infection, Davis hopes this newfound capability will yield tools to outsmart cancer cells, improve organ transplantation, and devise better vaccines.

Using a different imaging technology—positron emission tomography (PET)—to scan the immune system, HHMI investigator Owen N. Witte has also been able to visualize—and quantify—the generation of an immune response deep in the body. In his laboratory at the University of California, Los Angeles, Witte and his team used PET to detect radioactive chemical tracers in immune cells of mice with a solid tumor. The PET studies could track the immune response throughout the mice's bodies. T cells normally remain relatively inactive in lymph nodes, which serve as T-cell reservoirs, but in his PET studies, nodes even some distance from a tumor showed T-cell activity at least 10 times higher than normal levels.

The tracers enabled the scientists to observe specific immune cells as they sprang into action in response to the cancer. "This lets us see not only how but where" the body is responding to disease, Witte explains. Eventually, he believes, such PET scans could allow clinicians to observe the ebb and flow of the immune system over the course of a disease, such as cancer or an autoimmune disorder, and to evaluate the effectiveness of treatment.

A COMPETITIVE EDGE

Meanwhile, HHMI investigator Philippa Marrack, a onetime doubter of the benefits of video recordings of the immune system in action, has been converted. Her team at the National Jewish Medical and Research Center in Denver will soon begin recording T cells to probe a phenomenon they discovered. They found that T cells compete with each other for antigens on a type of antigen-presenting cell called a dendritic cell. Dendritic cells gather antigens in tissue and then carry them into lymph nodes where they activate the T-cell response.

Now her laboratory is going to use multiphoton microscopy to find out if the T cells' competition leads the "winning" T cell to deny other T cells access to the antigen. This may prove important to the design of multivalent vaccines, which are composed of two or more antigens to stimulate a broader response to infection or a response to more than one type of disease. By recording the immune response in action when two antigens are present, she hopes to determine whether T-cell competition is undermining the immune response to multiple antigens. If so, perhaps this competition needs to be taken into account when designing certain types of multivalent vaccines, particularly complex DNA vaccines such as those being developed against HIV.

According to Davis, "You always have more questions to ask than the current state of the technology is capable of answering." But he believes the broadening array of video imaging studies will eventually lay out the molecular choreography of the immune system. Knowing just which steps and missteps occur in that biochemical dance may be key in improving health for all—from developing new vaccines to helping the body rid itself of cancer cells. ■