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New Insight into How T Cells Ready for War

Howard Hughes Medical Institute researchers have discovered how the T cell, the body's chief immune-system warrior, organizes signaling molecules that mobilize the weaponry used to attack foreign invaders. The researchers said their basic findings contribute new knowledge that may aid in developing drugs that modulate the assembly of immune system molecules into functional complexes.

HHMI investigator Ronald D. Vale and colleague Adam Douglass at the University of California, San Francisco published their findings in the June 17, 2005, issue of the journal *Cell*.

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— Ronald D. Vale

"Historically, investigators thought about signaling pathways without much consideration of spatial organization," said Vale. "They were interested in which molecules "talk" to one another without taking into account that these molecules may need to be organized in particular spatial arrangements in the cell in order to work optimally." T cells have been important targets for such studies because they undergo critical reorganization of their structure and surface signaling molecules in order to coordinate an immune response, said Vale.

One popular theory, he said, has been that the components of this signaling machinery are organized by "lipid rafts"—islands of mutually attractive lipid molecules floating in the semi-liquid plasma membrane that marks the boundary of the cell.

"There is a popular idea that lipid rafts enable T cells to enhance immune responses by concentrating signaling molecules into functional subdomains,

rather than by having them move randomly to find one another within the plasma membrane. But most of the data derives from biochemical experiments rather than observations of living cells,” he said.

In their experiments, Douglass and Vale used live cell microscopy to determine whether lipid rafts within the plasma membranes indeed create such “microdomains” of signaling molecules. They used two types of microscopy to trace the movements of fluorescently labeled T-cell signaling molecules. One technique, called laser confocal microscopy, “gave us a sort of satellite view to see if there were concentrations of particular proteins in the plasma membrane—like staring down on the Pacific Ocean to spot islands,” said Vale. The other technique, called total internal reflectance fluorescence, enabled the researchers to zoom in on individual molecules to follow their movements in the plasma membrane.

The researchers spotted “islands” that were highly enriched in signaling molecules. They also tracked the movements of individual fluorescently-tagged signaling molecules whose biochemical characteristics suggested they would associate with lipid rafts as well as others that would not. “If these signaling molecules were free and unencumbered, they would bounce around rapidly and randomly in the membrane. But if they associated with a large, organized microdomain, then they would slow down, or even appear to be fixed in their position,” said Vale.

The researchers' experiments revealed that signaling microdomains that dotted the surface of the membrane were not lipid rafts, said Vale. “We saw individual molecules moving fast at times and becoming trapped in place in the membrane at other times. But which types of molecules partitioned into these stationary phases did not correlate with whether they were classified as lipid raft associated based upon biochemical criteria,” he said.

Douglass and Vale also developed a novel microscopy method of superimposing the tracks of single signaling proteins moving throughout the membrane with the “satellite” view which revealed the location of the “islands” of signaling molecules. Strikingly, they found that some proteins became trapped when they entered a signaling microdomain, but could later break free again and move throughout the membrane. Thus, the signaling microdomains are dynamic, with molecules coming and going. Other molecules appear to be excluded from these privileged microdomains, knocking at the door but being refused entry.

“We think that this stopping and starting behavior reflects signaling molecules joining the microdomains by forming protein-protein interactions, and then eventually breaking free from these connections,” said Vale.

Vale said their experiments suggest that molecular interactions among the proteins governed these associations and the creation of protein microdomains. “One of the proteins we followed, called LAT, is an adaptor protein,” he said. “It's like an octopus in that it has many domains that allow LAT to grab hold of several proteins at once. We found that cells that lack LAT have a greatly reduced ability to form microdomains.”

Vale emphasized that their findings do not rule out the existence or function of lipid rafts. “In different biological systems, lipid rafts may have importance,” he said. “Also, there are spatial and temporal limitations to our microscopy techniques. If lipid rafts are exceedingly small and short lived, as some people believe, then they would have been difficult to detect with the techniques we used.”

“However, we can say that a prominent mechanism for microdomain formation—and one that is quite obvious by these techniques—involves creating a network of protein-protein interactions,” said Vale. “Such mechanisms for creating signaling microdomains have been known for many years to exist in chemotactic bacteria, and they may be more prominent in creating microdomains of signaling molecules in mammalian cells than people have appreciated before.”

Insights into spatial organization are important for basic understanding of signaling, and perhaps will have clinical implications. “Spatial organization of these signaling molecules—that is, the ability to concentrate particular molecules and exclude others—could dramatically affect how signaling systems respond to external stimuli,” he said.

“And although it is totally speculative at this point, if we understood the principles of this spatial organization, we could potentially manipulate it for therapeutic purposes. However, we are now at a very early stage of understanding the spatial patterning of signaling molecules microdomains—for example, the inventory of molecules contained within these microdomains, how they are held together, and most importantly, their impact on cellular functions and signaling,” said Vale.