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Understanding Natural Killers Could Lead to New Hepatitis Treatments

Researchers have discovered that natural killer T (NKT) cells, the immune system's sentinels, patrol the labyrinthine blood vessels of the liver for invaders or signs of tissue damage and demonstrate a dogged behavior not seen before in other T cells.

The new studies show that NKT cells crawl along vessel walls, even upstream against blood flow. They halt only when they receive a chemical signal to unleash an immune-system assault on marauding microbes, other invaders or damaged tissue.

The findings offer a new way of thinking about this important class of immune cell, which is responsible for the inflammation and cell death in the liver due to hepatitis. Hepatitis can be a reaction to viruses, parasites such as malaria or other infections. Learning to "call off" the NKT cell's pursuit and attack could offer a treatment for hepatitis and associated complications.

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- Dan R. Littman

The researchers, led by Dan Littman, a Howard Hughes Medical Institute investigator at New York University (NYU) School of Medicine, published their findings online on April 5, 2005, in the *Public Library of Science Biology*. Lead authors on the paper were Frederick Geissman in Littman's laboratory and Thomas Cameron in the laboratory of co-author Michael L. Dustin, also of NYU. Other co-authors were from the La Jolla Institute for Allergy and Immunology and Millennium Pharmaceuticals in Cambridge, Mass.

Although it was known that NKT cells were more prevalent in the liver than in any other organ, said Littman, it was not known how they accomplish the

Herculean task of immune surveillance in the liver. The liver detoxifies and removes waste products from the blood. Inside the liver, vascular passages, or “sinusoids,” are filled with a witches' brew of nutrients, toxins, proteins, lipids and other chemicals. Thus, immune guardians that patrol the liver must tolerate many foreign molecules, yet respond readily to infection.

“It wasn't clear how NKT cells survey (tissue), or even if they survey at all,” said Littman. To visualize the activity of NKT cells in the liver, Geissman used mice in which NKT cells were tagged with a fluorescent marker. This was accomplished by replacing the gene encoding a characteristic NKT cell surface receptor called CXCR6 with the gene for green fluorescent protein. Although the CXCR6 receptor is known to be central to the function of NKT cells, its overall role was not known, said Littman.

Working with Geissman, Cameron adapted a technique called intravital fluorescence microscopy that enabled them to observe in real time the behavior of the tagged cells in the livers of mice.

“The startling discovery was that these NKT cells just move within the sinusoids intravascularly,” said Littman. By contrast, he said, immune cells in the lymph nodes and spleen perform their surveillance ensconced within specialized compartments shielded from the turmoil of the bloodstream. “In this case, it looks like NKT cells are doing their surveillance from within the vessels,” he said.

The observations revealed that the NKT cells crawl randomly within the sinusoids, even against blood flow, passing one another and even changing direction, said Littman. “It is very different from the kind of classical mechanism of lymphocytes rolling through vessels with the blood flow and when they are activated coming to a stop and then crossing through vessel walls in response to a signal.”

The researchers observed that the roving NKT cells stopped their movement when alerted by a foreign protein, called an antigen, “We think this is a reflection of their normal function of searching for antigen,” said Littman. “Whenever there is detection of antigen reflecting some kind of damage or local infection, the cell would stop in the vicinity of that signal and provide cytokine signals that would attract other inflammatory cells that destroy the invading microorganism and may also facilitate repair of the damage.”

In other experiments, the researchers explored the role of the CXCR6 receptor in the NKT cell's behavior. Receptors are protein sensors that nestle in the membranes of cells and detect external signaling molecules called ligands. When ligands are bound by the receptor, a specific chemical signal is transmitted to the interior of the cell.

In the case of NKT cells, the researchers found that the mice genetically rendered deficient in CXCR6 showed reduced survival of their NKT cells,

but no change in the speed or pattern of their patrolling. The studies showed that the presence of CXCR6 prolonged the NKT cells' survival. The researchers also found that the NKT cells of CXCR6-deficient mice showed a reduced patrolling, as well as a decreased severity of artificially induced hepatitis.

“So, all the evidence we can obtain so far points to CXCR6 being involved in promoting survival of these NKT cells when they get into the environment of the liver, and that's how the cells tend to accumulate there,” said Littman. “Our data don't support a critical role of CXCR6 in crawling behavior of the cells.”

Evidence for the role of CXCR6 in the survival of NKT cells—as well as the cells' involvement in triggering hepatitis—suggests a possible clinical implication of the findings, said Littman. “In general, these NKT cells could have an important inflammatory role, particularly in the case of chronic hepatitis,” he said. “If that is the case, we speculate that it may be possible to manipulate the NKT cell, perhaps by interfering with CXCR6 function, to ameliorate the inflammatory process,” he said.

Still unknown, said Littman, is which antigens alert NKTs to infections, as well as the nature of the regulatory machinery of crawling and stopping. The chemical the researchers used in their experiment is a general immune activator and does not reflect what occurs during an actual infection, he noted. Such knowledge would offer important insights into the mechanism of inflammation and liver damage due to infections, he said.