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Closing In on How Natural Killer Cells Thwart Viral Infection

Researchers have identified a receptor on the surface of natural killer cells in mice that is vital to resisting viral infection. The scientists' discovery offers new insights into innate immunity, a rapid response system that allows the host to fend off invading microorganisms until other arms of the immune system are mobilized.

In an article published in the May 4, 2001, issue of the journal *Science*, researchers led by Howard Hughes Medical Institute investigator Wayne M. Yokoyama report that they identified a receptor, called Ly-49H, that appears to seek out cells infected with murine cytomegalovirus (MCMV) and activates natural killer (NK) cells to attack those cells. The research team included Yokoyama's colleagues at Washington University School of Medicine in St. Louis and Anthony A. Scalzo at the University of Western Australia.

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"Natural killer cells are best known for their capacity to kill tumor cells before they become established cancers," said Yokoyama. "But there has also long been evidence for their role in controlling infection in the earliest phases of the body's immune response.

"However, a central issue has been how natural killer cells respond to pathogens," he said. "While there have been data suggesting that they respond to cytokines made by other innate immune cells, such as macrophages, our hypothesis has been that NK cell receptors involved in tumor killing are also involved in antiviral defense. In this paper we've identified such a receptor for the first time that appears to be vitally involved in host defense against a virus," said Yokoyama.

The scientists began their search for the receptor by exploring the region, termed the NK gene complex (NKC), on mouse chromosome 6 that was known to contain a large cluster of genes for receptors found on NK cells.

Co-author Anthony Scalzo of the University of Western Australia had previously identified the locus, which is called the *Cmv1* resistance gene, that controls resistance to MCMV.

Initially, Yokoyama and his colleagues had no clue about which receptor they were looking for because the NKC region contained so many genes. "The NK gene complex has many receptors, most of which are orphan receptors because their exact physiologic function and what they recognize is not clearly understood," he said. "We actually had too many candidate genes."

To make matters worse, NK cells are controlled by the interaction of inhibitory and activating receptors, so the search for the receptors was made even more difficult. Fortunately, however, Yokoyama and his colleagues identified a strain of mice at The Jackson Laboratory that aided them in their search for the NK receptor.

This mouse strain, called BXD-8, was produced through a series of matings that resulted in a strain that was susceptible to MCMV, even though the mouse still had a large portion of the NKC from a virus-resistant mouse. "Based on the genetics, we would have predicted it should be resistant to the virus, but it was susceptible," said Yokoyama.

After additional genetic studies, the researchers suspected that the cause of viral susceptibility was a single defective gene within the NKC region. "Those findings gave us a target, and we thought that if we could identify the gene that was selectively disrupted in the BXD-8 mice, then we would find the gene that normally confers resistance," said Yokoyama.

By testing a large number of monoclonal antibodies known to bind specific NK receptors, the researchers finally determined that the BXD-8 mouse NK cells failed to show reactivity only to a monoclonal antibody specific for Ly-49H. Other analyses confirmed that the mice were not producing messenger RNA for the Ly-49H protein due to a structural abnormality in the Ly-49H gene. Immunological studies with mice that are normally resistant to the virus showed that injecting antibodies against Ly-49H rendered them susceptible to the virus.

"We have shown that Ly-49H is critically involved in resistance to mouse cytomegalovirus," concluded Yokoyama. "In the genetic absence of the receptor, or when we inject an antibody that appears to block the receptor's recognition function, the animals succumb to viral infection, as evidenced by high viral replication and lethality."

According to Yokoyama, the discovery of Ly-49H and its function also suggests that NK cells may be more closely related to B and T cells than has been previously appreciated. "The receptor system and activation cascade that NK cells seem to use in this aspect of anti-viral defense is different from the one that macrophages use in innate immunity," said Yokoyama. "It's an irony that, even though NK cells are involved in innate immunity, and not in specific immunity, they use the same or a similar signaling pathway to that used by specific B and T cells."

The identification of Ly-49H represents the beginning of what Yokoyama hopes will be a promising model system in which to study the activation of NK cells in an important clinical area. Future studies by Yokoyama and his colleagues will explore the responsiveness of Ly-49H to other viral infections and exactly how it recognizes viral-infected cells to trigger NK cell attack.

Basic understanding of NK cell activation could aid in the development of new clinical approaches to enhancing resistance to viruses, said Yokoyama. "Viruses such as CMV are particularly dangerous for AIDS patients or for individuals who are immunocompromised, such as those who are receiving a bone marrow transplant or those who are being immunosuppressed for treatment of other diseases," he said.