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Rare Childhood Blood Disorder Sidesteps Immune System's Attack

As the assassins of the immune system, natural killer cells hunt down renegade cells that have been corrupted by viruses and broken genes. Now, Howard Hughes Medical Institute international research scholar André Veillette and his colleagues have learned how a molecular switch helps to prevent cancers by signaling natural killer cells to attack abnormal blood cells.

The findings, published on August 2, 2009 in *Nature Immunology*, may also lead to better understanding of a rare genetic disorder called X-linked lymphoproliferative disease (XLP), which causes the immune system to go into overdrive. Children who have the genetic defect that causes XLP face a greater risk of severe complications from viral infections.

For example, healthy children who become infected with Epstein-Barr virus may develop mononucleosis, an illness that usually lasts several weeks. But Epstein-Barr infection can prove fatal in children with XLP. Their immune cells pile up and clog the lymph nodes, spleen, and liver. In the 1970s, clinicians noted that the blood cancer lymphoma was also very common in boys with XLP. "If you don't treat these children, they die," said Veillette, who is based at the Clinical Research Institute of Montreal (IRCM) and the University of Montreal.

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- André Veillette

In the late 1990s, researchers found that XLP resulted from mutations in a gene that makes a small protein called SAP, one of a family of molecules that is abundant in many immune cells, including natural killer cells.

"When people found the SAP mutations in these patients, they realized that the mutations led to a lack of SAP, which in turn caused defects in many

types of immune cells, such as T cells, B cells, and natural killer cells,” Veillette says. “It’s likely that the combination of all these immune defects causes the symptoms of the disease.”

Veillette decided to study natural killer cells because they carry an array of odd antennae, or receptors, that sense whether other cells are normal or abnormal. When those antennae sense an abnormal cell—like those with defects that cause many types of cancers, including blood cancers—natural killer cells attack by dumping a flood of chemicals that perforate and destroy their target. “They’re very good at doing this. They’re on a hair trigger, and they do it very quickly,” Veillette says.

About five years ago, Veillette took a close look at SLAM receptors, one of the many types of receptors on the surface of natural killer cells. Other researchers had discovered that these

molecules interact with SAP (which stands for SLAM-Associated Protein), but no one understood what role SAP played in commanding the immune system’s assassins to attack.

To find out, Veillette genetically engineered mice that could not produce the SAP family of molecules, including SAP and two closely related molecules, EAT-2 and ERT. These triple knock-out mice developed normally and appeared to possess healthy immune cells. However, when Veillette put natural killer cells from the mice into Petri dishes, he discovered something odd. The natural killer cells could destroy some types of cancer cells, but they were unable to target and attack cancerous blood cells. Further experiments in live mice confirmed that the natural killer cells lacking the SAP-related molecules were unable to kill lymphoma cells.

“These cells exhibit a very specific defect,” Veillette says. “They are unable to eliminate abnormal hematopoietic (blood-forming) cells. But they can kill other bad cells like colon carcinoma cells and melanoma cells.”

“This finding was unexpected,” he continues. “We knew there would be a defect, but we didn’t realize it would be specific to the blood system.”

The new research fills a critical gap in understanding how the SLAM receptors work. SLAM receptors are found on the surface of most blood cells, including natural killer cells. SLAM receptors on the surface of a natural killer cell will recognize SLAM receptors on the surface of, for example, a cancerous blood cell — but only if other receptors on the natural killer cells that signal the cell is abnormal are also active. This combination tells the natural killer cells to attack.

When this “kill” signal is triggered, the SLAM receptors on the target cell stick into the surface of the natural killer cell, which activates the SAP proteins inside the natural killer cell. “The SAP proteins allow the SLAM

receptors to work,” Veillette says. “When you don't have SAP, as in our mice, the activating capacity of the SLAM receptors completely disappears.” His team is still working out the details of what SAP does inside the cells.

Veillette and his colleagues also found that the absence of SAP-related molecules actually flips the signal sent by the SLAM receptors. Instead of triggering a “kill” response, the receptors send a “don’t kill” signal. And the researchers found that mice that cannot produce SAP readily accumulate cancerous blood cells in their body—as happens in children with XLP—because the animals’ immune system can no longer destroy mutated white blood cells.

Veillette says his findings help explain the uncontrolled immune reaction that occurs during viral infections in children with XLP. Normally, if SAP were present, the immune system would clear the spent white blood cells that rush to the lymph nodes, the spleen, and other sites of infection. But without SAP, white blood cells pile up, causing serious inflammation.

The standard treatment for XLP is bone marrow transplantation, and that’s unlikely to change, Veillette says. However, he thinks future research on SAP-related molecules could point toward better therapies for other viral infections or autoimmune diseases. To that end, Veillette is now developing mouse models to further examine the role of SAP during these conditions.

"SAP has really been a mystery,” Veillette says. “It's nice to begin to understand the impact of this little molecule that has puzzled a lot of people."