

## Frog secretions block HIV infections

By Leigh MacMillan

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A new weapon in the battle against HIV may come from an unusual source – tropical frogs. Investigators at Vanderbilt University Medical Center that compounds secreted by frog skin are potent blockers of HIV infection.

The findings, reported this month in the *Journal of Virology*, could lead to topical treatments for preventing HIV transmission and reinforce the value of preserving the Earth's biodiversity.

“We need to protect these species long enough for us to understand their medicinal cabinet,” says Louise A. Rollins-Smith, associate professor of microbiology & immunology, who has been studying the antimicrobial defenses of frogs for about six years.

Frogs, she explains, have specialized granular glands in the skin that produce and store packets of peptides, small protein-like molecules. In response to skin injury or alarm, the frog secretes large amounts of these antimicrobial peptides onto the surface of the skin to combat pathogens like bacteria, fungi and viruses.

Rollins-Smith happens to have the laboratory next door to Derya Unutmaz, associate professor of microbiology & immunology. During a hallway chat one day, the two decided it would be interesting to investigate whether any frog peptides have activity against human viruses, specifically HIV, the focus of Unutmaz's group.

Postdoctoral fellow Scott E. VanCompernelle screened 15 antimicrobial peptides from a variety of frog species for their ability to block HIV infection of T cells, immune system cells targeted by HIV. He found several that inhibited HIV infection without harming the T cells.

The peptides appear to selectively kill the virus, perhaps by inserting themselves into the HIV outer membrane envelope and creating “holes” that cause the virus particle to fall apart, Unutmaz said.

“We like to call these peptides WMDs – weapons of membrane destruction,” Unutmaz quips. It is curious that the antimicrobial peptides do not harm the T cells at concentrations that are effective against the virus, he notes, since HIV’s outer membrane is derived from, and therefore essentially identical to, the cellular membrane. The investigators have proposed that the peptides act selectively on the virus in part because of its small size relative to cells.

The ability of the peptides to destroy HIV was enticing, but to be really effective as antimicrobial agents, they need to prevent transmission of HIV from dendritic cells to T cells, Unutmaz said.

Dendritic cells, he explains, are the sentinels of the immune system. They hang out in the mucus-generating surface tissues, scanning for invading pathogens.

“Their purpose in life is to capture the enemy, bring it to the lymph node – the command center – and present it to the general, the T cell, to activate a battle plan,” Unutmaz says. “It’s a very efficient system that has allowed us to survive many insults, pathogens, and viruses.”

But HIV is a wily foe. When it is picked up at the mucosal surface by a sentinel dendritic cell, it somehow evades destruction. Instead, it hides inside the cell, waiting to invade the T cell with a Trojan Horse-like mechanism. The ability of HIV to remain hidden in the dendritic cell, avoiding destruction by circulating antibodies and immune system cells, “may explain why after 20 years we don’t have a vaccine for this virus,” Unutmaz says.

To test the effectiveness of the frog peptides in preventing HIV transmission, VanCompernelle first allowed cultured dendritic cells to capture active HIV. He then incubated the HIV-harboring dendritic cells with antimicrobial peptides, washed the peptides away, and added T cells.

“Normally the dendritic cell passes the virus to the T cell, and we get very efficient infection of the T cell,” Unutmaz says. “But when we treated the dendritic cells with peptides, the virus was gone, completely gone. This was a great surprise.”

The finding was puzzling, he explains, since the prevailing notion is that HIV captured by dendritic cells is hidden and protected. The investigators currently are using imaging technologies to test the hypothesis that HIV is actually cycling to the dendritic cell surface.

“We think maybe it’s popping its head out, looking around for a T cell, and then going back inside to hide until it cycles out again,” Unutmaz said. If peptide is present outside the cell, “it targets the virus that pops up and kills it.” Preliminary experiments suggest that the hypothesis is correct.

“This is very exciting, as it suggests that these peptides could be very effective since the virus now has nowhere to hide,” Unutmaz says. “And if this cycling is really happening, we may be able to generate a vaccine that will target virus captured by dendritic cells.”

The frog peptides are an exceptional tool for probing “what the virus knows about the dendritic cell that we don’t know,” Unutmaz added. “How does HIV manage to survive and cycle back and forth to the cell membrane? If we can understand that, we’ll find the gaps, and that will open a whole new universe of targets for intervention.”

The investigators learned this week that the American Foundation for AIDS Research will fund their continuing quest to understand how the frog peptides kill HIV in dendritic cells. Their plans include imaging how the peptides work, screening additional frog peptides for activity, and testing peptides on a mucosal cell system to study the feasibility of developing them as prophylactics against HIV infection.

“If we are able to learn the mechanisms these peptides are using to kill HIV, it might be possible to make small chemical molecules that achieve the same results,” Unutmaz says. Such chemicals would be more practical as therapeutic microbicides.

“This study is a great example of how collaboration across disciplines leads to big discoveries,” Unutmaz says.

Other members of the department of microbiology and immunology assisted the investigators by providing viruses for testing. The team found that membrane-coated viruses were susceptible to destruction by the frog peptides, but non-coated viruses, such as reovirus and adenovirus, were not affected.

R. Jeffery Taylor, Kyra Oswald-Richter, Jiyang Jiang, Bryan E Youree, Christopher R. Aiken and Terence S. Dermody at Vanderbilt are co-authors of the study. The research was supported by the National Institutes of Health, the Elizabeth B. Lamb Center for Pediatric Research and the National Science Foundation.

### **Delving into the mysteries of declining frog populations**

Frogs around the world are in trouble. And as species are lost, so are their biological treasures.

The National Science Foundation has awarded a team of Vanderbilt University Medical Center investigators a four-year grant to study amphibian declines in Central America and California.

“Amphibian skin has long been favored in folklore for its medicinal properties,” says Louise A. Rollins-Smith, associate professor of microbiology & immunology and principal investigator of the new grant. “Frogs are a rich source of potentially useful molecules that might work against human pathogens.”

Rollins-Smith collaborated with Derya Unutmaz, M.D., assistant professor of Microbiology & Immunology, and other Vanderbilt scientists to show this month that compounds from frog skin block HIV infection.

Frogs produce and secrete compounds called antimicrobial peptides to fight off bacteria, fungi and viruses that land on their skin, Rollins-Smith explains.

“Frogs have evolved over millennia to combat such pathogens, so we want to learn from the frog as much as we can about these molecules,” she says.

With the new grant, Rollins-Smith and her team will be investigating the antimicrobial defenses of declining frog populations that are facing a particular skin fungus. Postdoctoral fellow Douglas C. Woodhams will be traveling to sites in Panama and in California to collect samples of the skin peptides from affected frogs.

“Our goal is to study frog populations that are ahead of an epidemic of this fungus, and those that are behind an epidemic to see if the ones that have survived have beneficial protective peptides,” Rollins-Smith says.

The [Mass Spectrometry Research Center at Vanderbilt](http://www.mc.vanderbilt.edu/root/vumc.php?site=msrc)

[<http://www.mc.vanderbilt.edu/root/vumc.php?site=msrc>] is particularly valuable to the team’s studies. Using mass spectrometry, it is possible to characterize the array of peptides in the samples and rapidly focus on and sequence those that might be antimicrobial.

“We hope to figure out which species are most vulnerable to this fungal pathogen so that they can be the focus of greater conservation efforts,” Rollins-Smith says.

The studies may also reveal new antimicrobial peptides which could be useful blockers of human pathogens, she added.