Circle of protection against HIV

By Michael J. Haas, Senior Writer

New *in vitro* research suggests that HIV infection can be prevented by inducing the endogenous production of θ -defensins, a class of cyclic antimicrobial peptides whose expression is thought to be blocked in the vast majority of humans.¹ The use of aminoglycoside-based vaginal microbicides to induce the peptides is being tested for safety in macaques.

θ -Defensins are a class of cyclic 18-amino-acid peptides that contain 3 disulfide bonds and target HIV entry into cells. The compounds were first identified in rhesus macaques in 1999,² but thus far nobody has found them to be expressed in humans. A 2002 study by a **University of California, Los Angeles (UCLA)** team found that humans have *defensin- θ (DEFT)* genes, but premature termination codons block their complete translation.³

The team synthesized the human θ -defensin peptides and showed that these molecules, dubbed retrocyclins, were able to protect human cells from HIV infection when added to the cell cultures.

In light of these studies, Alexander Cole of the **University of Central Florida (UCF)** and colleagues wanted to see whether inducing θ -defensin production in the human cells themselves would result in the cells being able to combat HIV infection on their own. The group turned to aminoglycoside antibiotics because they are known to suppress premature termination codons and enable translation of otherwise blocked genes.

Indeed, the team found that HIV-infected cells treated with the aminoglycoside antibiotic tobramycin, gentamicin or amikacin produced retrocyclins and showed decreases in viral loads compared with untreated controls.

The antibiotics also induced retrocyclin production in human cervicovaginal cell culture without causing adverse metabolic or toxic effects. The team published the data in *PLoS Biology* and included researchers from UCLA and the **Centers for Disease Control and Prevention**.

According to Cole, the focus is now “on aminoglycosides as topical microbicides, and conducting safety and toxicity studies in pigtail macaques.” Cole is an associate professor of molecular biology and microbiology at UCF. He is collaborating on these follow-up studies with Dorothy Patton, professor of obstetrics and gynecology at the **University of Washington**.

“What I like about this paper is that it established the importance of the innate immune system” to ward off HIV infection, said Joseph Romano, executive director for R&D at **The International Partnership for Microbicides (IPM)**, a not-for-profit organization.

He added: “A lot of us in the microbicide field take a drug development perspective—we focus on drugs or devices that we can put into the host. These researchers are looking at HIV prevention from a host perspective: instead of using an antiviral drug as a microbicide, they used a compound that could induce and empower the host’s immune system.”

IPM has completed Phase I studies of gel-based and vaginal ring-based formulations of dapivirine as microbicides to prevent HIV infection. Romano said IPM plans to start multiple Phase I/II studies of both formulations later this year. Dapivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI).

Mucosal barrier

The track record of aminoglycoside antibiotics suggests it will be particularly important to look at safety, as the compounds are known to cause severe renal toxicity and ototoxicity when given systemically to treat severe bacterial infections.

Thomas Moench, president and COO of **ReProtect Inc.**, also noted that although aminoglycosides have low bioavailability from the GI tract, their bioavailability through vaginal mucosae is unknown. This raises the possibility that topical microbicide use could lead to

systemic toxicity, he said.

Cole noted that studies by other groups suggest that the antibiotics do not penetrate beyond the mucosal barrier.

In addition to wanting data on potential systemic toxicity, researchers also wanted to know about the effect aminoglycoside microbicides have on bacteria in the vagina and lower GI tract.

“Long-term and continuous use by millions of women would constitute pressure toward antibiotic resistance by bacteria exposed to these antibiotics,” said Kevin Whaley, VP of strategic development at ReProtect. This might not be a significant problem if only vaginal mucosae are exposed to the aminoglycosides, “but resistance by gastrointestinal and other flora is a risk that will need to be assessed early on.”

IPM’s Romano said it would be good to know whether aminoglycosides have adverse effects on beneficial vaginal flora.

Cole said his team will investigate the effects of the aminoglycosides on such vaginal flora. He added that other researchers have shown that certain aminoglycosides do not adversely affect *Lactobacilli*, a genus of vaginal and gastrointestinal bacteria that create an acidic environment that inhibits the growth of pathogenic bacteria.

Bypassing antibiotics

Cole acknowledged that the potential for aminoglycoside microbicides to drive bacterial resistance needs to be considered. If the resistance

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—Joseph Romano,
International Partnership
for Microbicides

issue proves too formidable, he suggested that “nonantibiotic agents that can suppress premature termination codons—for example, PTC-124—might be useful alternatives.”

PTC-124 (ataluren) is a small molecule from partners **PTC Therapeutics Inc.** and **Genzyme Corp.** that facilitates the complete translation of proteins containing nonsense mutations. Ataluren is in a pivotal Phase IIb trial to treat the 15% of Duchenne muscular dystrophy (DMD) patients with a nonsense mutation in the gene encoding dystrophin, and it has also completed a Phase IIa trial in cystic fibrosis (CF). PTC declined to comment on whether ataluren had potential as a microbicide.

ReProtect's Whaley thinks the nonantibiotic route might be the way to go. “An alternate approach would be to seek other compounds with the desired effect on θ -defensin expression but without the antibiotic activity and toxicity of the aminoglycosides,” he said.

Whaley added that such alternative compounds might also be used to induce retrocyclins on a systemic level and thereby treat—not just prevent—HIV infection.

In February, ReProtect's BufferGel spermicidal microbicide consisting of a high molecular weight, cross-linked, buffering polymer, failed in a Phase II/Ib trial to prevent HIV infection. The company is now focused on developing Duet, its cervical barrier and applicator technology for vaginal delivery of spermicides, microbicides and therapeutics.

David Craik, professorial research fellow at **The University of Queensland's** Institute for Molecular Bioscience, had a different suggestion altogether: direct systemic administration of retrocyclins.

Craik said the peptides' cyclic nature and disulfide bonds made

them more metabolically stable than linear peptides. “So cyclic peptides like retrocyclins should have longer *in vivo* half-lives than linear peptides” and thus should bypass one of the chief hurdles of using peptides as therapeutics, he said. Craik led a team that solved the structure of θ -defensin in 2001.⁴

Cole said a patent application for the findings reported in *PLoS Biology* has been submitted.

He added that UCF and UCLA hold existing patents for the use of retrocyclins as antiviral and antibacterial agents and said those patents are available for licensing.

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REFERENCES

1. Venkataraman, N. *et al.* *PLoS Biol.*; published online April 28, 2009; doi:10.1371/journal.pbio.1000095
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2. Tang, Y. *et al.* *Science* **286**, 498–502 (1999)
3. Cole, A. *et al.* *Proc. Natl. Acad. Sci. USA* **99**, 1813–1818 (2002)
4. Trabi, M. *et al.* *Biochemistry* **40**, 4211–4221 (2001)

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