Infectious fatigue

By Lev Osherovich, Senior Writer

Chronic fatigue syndrome has eluded a specific molecular definition for decades, leading many clinicians to dismiss the ailment as psychosomatic or fictitious. Now, a study in *Science* points to a potential culprit in CFS—a recently discovered retrovirus that also has been implicated in prostate cancer.¹

The challenge will be proving that infection by xenotropic murine leukemia virus-related virus (XMRV) indeed causes CFS. In the absence of a workable animal model, confirmation of the *Science* report will require larger epidemiological studies.

CFS affects up to 17 million people worldwide and presents as a heterogeneous syndrome of exhaustion, muscle weakness and immunological deficiencies.² CFS's clinical similarity to other syndromes such as fibromyalgia, combined with a lack of biomarkers, has made it difficult to clearly identify patients for either epidemiological studies or clinical trials.

If the causative link between XMRV and CFS is borne out, the *Science* study, from a team led by Judy Mikovits at the **Whittemore Peterson Institute for Neuro-Immune Disease** and collaborators at the **Cleveland Clinic** and the **National Cancer Institute**, could open the door to a definitive diagnostic for the condition.

In addition, validation would then point researchers toward antiretrovirals as a potential therapeutic avenue for the condition.

"The main punch line is that this is a relatively new human pathogen," Mikovits told *SciBX*. "Clearly there's now a biomarker than can be used in clinical trials."

The Whittemore Peterson Institute is a not-for-profit foundation focused on CFS and other disorders involving immunity and the nervous system.

Burden of proof

XMRV was first identified in 2006 when researchers at the **University of California, San Francisco** found traces of XMRV in prostate tumors.³

Subsequently, spurred by earlier reports of compromised antiviral immunity in CFS patients, Mikovits' team isolated DNA from the peripheral blood mononuclear cells (PBMCs) of CFS patients and healthy controls. The team found traces of XMRV's genome in the isolated DNA of 68 out of 101 CFS patients but in the DNA of only 8 of 218 healthy controls.

The team then probed the PBMCs from 30 patients and 5 controls using a panel of antibodies that detect proteins from animal viruses related to XMRV. They found that PBMCs from 19 of the CFS patients had viral proteins, whereas none of the PBMCs from control patients did.

Mikovits' team then showed that virus from CFS patients could spread *in vitro*.

"I think this article is as solid as you get for a first report of this kind," said John Coffin, professor of molecular biology and microbiology at **Tufts University** who coauthored a perspective piece that accompanied the *Science* article. "A lot of additional papers will be needed to establish causality, but for a first step this is really solid."

In fact, whether XMRV plays a causative role in either CFS or prostate cancer will likely require years of preclinical validation (see **Box 1, "Prostate cancer connection"**).

At this point, not all researchers are convinced that Mikovits' findings conclusively show a connection between XMRV and CFS. Donald Ganem, professor of microbiology and immunology at UCSF, raised concerns about the study's cohort of patients as well as the analytical procedures used to identify XMRV.

Ganem, a co-discoverer of XMRV, said there has been a long history of attributing CFS to various viruses, none of which has been borne out in subsequent analyses.

"XMRV is the latest in a long series of candidates," said Ganem.

Previously, other pathogens such as Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6) had been implicated in CFS but definitive proof of their involvement in the disorder has been elusive. In 2004, the Whittemore family founded the **HHV-6 Foundation** to investigate that virus' role in CFS.

Mikovits said that her team plans to test how XMRV infection influences susceptibility to HHV-6 and other viruses previously implicated in CFS.

Ganem added that the *Science* paper "is difficult to evaluate because the patients and the control groups are not described demographically. You can't tell in the paper if they're even matched for age and sex."

Ganem also noted that the large amount of amplification used in PCR tests to identify the virus creates a risk of false positives due to amplification of even tiny amounts of sample cross-contamination.

Also, he said, the level of infection in positive subjects, as revealed by the antigen detection tests in PBMCs, seemed surprisingly high for a natural human infection.

"It would be very exciting if true, but until this paper is confirmed, I'm still on the fence," he said.

According to Mikovits, the samples were drawn from a specimen library accumulated over many years by the Whittemore Peterson Institute from age, sex and zip code-matched patients and controls who had been diagnosed with CFS by clinical criteria such as exhaustion and immune dysfunction. She noted that her team's antibody-binding studies were consistent with the PCR results and thus were not likely to be due to sample cross-contamination.

"A lot of additional papers will be needed to establish causality, but for a first step this is really solid."
—John Coffin, Tufts University

Box 1. Prostate cancer connection.

Although findings published in *Science* posit a potential link between xenotropic murine leukemia virus-related virus and chronic fatigue syndrome, a study in the *Proceedings of the National Academy of Sciences* strengthens the argument that XMRV could cause prostate cancer.⁴ That report, from a Utah team, provides evidence that XMRV is more common in prostate tumors than previously thought.

An earlier study by researchers at the **University of California, San Francisco** identified XMRV in the non-cancerous stromal tissue surrounding prostate tumors.³ However, the study used a small number of samples and did not examine healthy prostate tissue.

In the new report, senior author Ila Singh and co-workers tested prostate samples from 233 prostate cancer patients and 101 healthy controls for molecular signatures of XMRV. The study “shows that this virus has an association with cancer,” she said.

Although the UCSF study identified XMRV in nearby cells, Singh said her

team found that “the virus is present in malignant cells, which is very important for showing that it could cause cancer.”

Singh is an associate professor of pathology at **The University of Utah**.

Indeed, her team found XMRV DNA in 6.2% of prostate cancer samples and in 2% of healthy controls. When the team probed the samples with an antibody that detects XMRV protein, 23% of the prostate tumors had viral proteins compared with 4% of control tissue.

Singh attributed the discrepancy between the DNA and protein detection methods to the antibody’s higher sensitivity compared with that of the PCR technique used to detect viral DNA.

Donald Ganem, professor of microbiology and immunology at UCSF and an author of the original XMRV study, said the new findings appear to conflict with the earlier findings about which cells are infected by XMRV. However, he said doubts about the Utah team’s techniques and reagents will need to be addressed in future studies.

“It’s interesting that in Singh’s paper the prevalence of XMRV was higher by immunohistochemistry than by PCR,” said Ganem. “That’s unusual and raises questions about the specificity of the antiserum.”

Singh’s next step is to show that XMRV infection can actually lead to cancer. Although there are no good mouse models of prostate cancer, she thinks the virus could potentially cause other tumors if it integrates into the genome and activates oncogenes.

“It’s not so important to cause prostate cancer specifically,” said Singh. “If you get any kind of cancer it would be an important step.”

To test her hypothesis, Singh plans to inject the virus into mice that harbor human immune cells, which are prone to XMRV infection, and then test the mice for lymphatic tumors.

Meanwhile, she has filed provisional patents on the diagnostic methods described in the *PNAS* study. The IP is available for licensing. —LZO

To prove that a pathogen causes a disease, epidemiologists have traditionally relied on criteria postulated by 19th-century microbiologist Robert Koch. These criteria—still considered the gold standard—include being able to isolate the pathogen *in vitro* and using this material to infect healthy hosts.

The problem is that it’s difficult to meet these criteria. “Unless you have an animal model, you don’t have the opportunity to fulfill Koch’s postulates,” said Ganem. “You then have to rely on epidemiological criteria to determine causality.”

“Technically, with this virus you can’t satisfy Koch’s postulates,” said Mikovits, in part because there is no animal model.

Mikovits wants to examine a larger population of healthy controls and CSF patients to establish a tighter link between CFS and XMRV infection. A similar approach was used in the 1980s to shore up the connection between HIV and AIDS.

“Just like you can’t have AIDS without having HIV, if we show that you can’t have CFS without having XMRV” it would bolster the case for causality, said Mikovits.

She also noted that it’s unknown whether the 3.7% of healthy individuals who tested positive for the virus are perhaps undiagnosed patients. It’s also unclear why roughly one-third of CFS patients tested negative.

Coffin said proving causality would require “epidemiological studies to firm up the association between infection and disease and

temporal studies to look at the onset of disease upon infection.”

“The test of causality will be a larger epidemiological study,” agreed Robert Silverman, professor of cancer biology at the **Cleveland Clinic Lerner Research Institute** and a coauthor of the *Science* paper. Silverman was also a coauthor on the 2006 paper that first described XMRV.

Animal model needed

Currently, there is no disease-modifying therapy for CFS. In light of their discovery, Silverman and Mikovits are optimistic about the prospects for a direct antiviral strategy to treat the disorder.

“If the virus is the cause of CFS and you stop the virus, you could cure the disease,” said Silverman.

The challenge is to identify the right antivirals. Silverman thinks screening known antivirals in a cell culture assay of XMRV replication could be a good way to identify lead candidates. “The virus grows very well in cell culture, so it’s pretty easy to determine inhibitory doses,” he said.

However, antivirals that are effective against XMRV may need to be different than those used to treat other viral infections because XMRV belongs to a class of slow-growing retroviruses. According to Mikovits and Silverman, conventional antivirals such as reverse transcriptase inhibitors that work by inhibiting viral replication may not work as well against XMRV as they do against HIV. Indeed, the

serum of infected individuals has low levels of virus, suggesting XMRV replicates infrequently, unlike HIV.

Mikovits said the viral protease needed for XMRV's life cycle is fairly different from that of HIV, making that class of approved antivirals potentially less useful.

Lawrence Blatt, president and CEO of **Alios BioPharma Inc.**, also thinks antiviral compounds developed for other indications are unlikely to be effective.

Alios, which was cofounded by Silverman, is developing a broad-acting compound that activates RNase L, an antiviral component of the immune system.

"Although related viruses share life-cycle strategies, they have disparate sequences. I wouldn't necessarily say that other antiretroviral drugs will work against XMRV," said Blatt.

He also noted that protease inhibitors for HCV do not work against related viruses like West Nile virus.

Thus, Blatt thinks developing an animal model for XMRV infection would not only shore up the case for causality but would also enable drug screening efforts.

"An animal model in which the virus causes infection would be the smoking gun," he said.

Mikovits and colleagues "have shown that XMRV has infectious qualities in cell culture, so it's quite conceivable they could use a transgenic mouse to assess the clinical characteristics of the disease that are affected by the virus," said William Carter, chairman and CEO of **Hemispherx Biopharma Inc.**

Hemispherx's Ampligen, an RNA-based immunomodulatory compound, is under **FDA** review to treat CFS, in which its mechanism of action is unknown.

The FDA missed Ampligen's May 25 PDUFA date, and Carter said the company is in the process of submitting additional preclinical toxicology and manufacturing data to the agency.

Daniel Peterson, a coauthor on the *Science* study and a founder of the Whittemore Peterson Institute, is the principal investigator in clinical trials of Ampligen. Carter said the company was not involved in the *Science* study.

Diagnostic beliefs

If the findings reported in *Science* bear out in larger studies, the most immediate application of the discovery may be to establish a molecular

"An animal model in which the virus causes infection would be the smoking gun."

—Lawrence Blatt,
Alios BioPharma Inc.

diagnostic for CFS.

"The discovery of this virus could make ambiguities of diagnosis go away," said Blatt. "XMRV infection would be an entry criterion into a diagnosis of CFS."

Being able to track XMRV infection may also answer questions about the virus' mode of transmission and could resolve whether and how CFS is infectious.

"There's some evidence of an infectious component to CFS in families or people who are closely confined with those who are ill," said Carter. "The mode of transmission is the \$64,000 question—it's up for grabs whether it's biological fluids, sexual contact, sharing foods, etc."

Silverman said he has previously filed patents on the detection of XMRV in prostate tumors and has out-licensed them to **Abbott Laboratories**.

Meanwhile, Mikovits has filed to patent other diagnostic procedures described in the *Science* study. These methods and reagents are available for licensing from the Whittemore Peterson Institute.

Osherovich, L. *SciBX* 2(40); doi:10.1038/scibx.2009.1492
Published online Oct. 15, 2009

REFERENCES

1. Lombardi, V.C. *et al. Science*; published online Oct. 8, 2009; doi:10.1126/science.1179052
Contact: Judy A. Mikovits, Whittemore Peterson Institute for Neuro-Immune Disease, Reno, Nev.
e-mail: judym@wpinstitute.org
2. Devanur, L.D. & Kerr, J.R. *J. Clin. Virol.* **37**, 139-150 (2006)
3. Urisman, A. *et al. PLoS Pathog.* **2**, e25; published online March 31, 2006; doi:10.1371/journal.ppat.0020025
4. Schlager, R. *et al. Proc. Natl. Acad. Sci. USA*; published online Sept. 7, 2009; doi:10.1073/pnas.0906922106
Contact: Ila R. Singh, The University of Utah, Salt Lake City, Utah
e-mail: ila.singh@path.utah.edu

COMPANIES AND INSTITUTIONS MENTIONED

Abbott Laboratories (NYSE:ABT) Abbott Park, Ill.
Alios BioPharma Inc., South San Francisco, Calif.
Cleveland Clinic, Cleveland, Ohio
Cleveland Clinic Lerner Research Institute, Cleveland, Ohio
Food and Drug Administration, Silver Spring, Md.
Hemispherx Biopharma Inc. (NYSE-A:HEB), Philadelphia, Pa.
HHV-6 Foundation, Santa Barbara, Calif.
National Cancer Institute, Frederick, Md.
Tufts University, Boston, Mass.
University of California, San Francisco, Calif.
The University of Utah, Salt Lake City, Utah
Whittemore Peterson Institute for Neuro-Immune Disease, Reno, Nev.