

New Directions

HHMI investigators find promising avenues for treating two diseases.

GALLSTONES: A GENETIC LINK

Cholesterol gallstone disease (CGD) afflicts about 20 million people a year, causing them considerable pain and often necessitating surgery. But new studies may point the way to some relief.

Researchers have found an experimental

compound that prevents CGD in mice by activating the biochemical pathway known to stimulate the liver's bile-acid secretion. In addition to indicating new approaches for the development of preventive drugs, their results suggest novel strategies for identifying people with a genetically increased risk for forming gallstones.

HHMI investigator David J. Mangelsdorf and his colleagues HHMI research associate Antonio Moschetta and Angie L. Bookout at the University of Texas Southwestern Medical Center at Dallas published their findings in the December 2004 issue of *Nature Medicine*. "What we saw was remarkable," says Mangelsdorf. "After just 5–7 days of treatment, these animals, which were on a diet that would normally produce cholesterol gallstone disease, showed no trace of the disease."

Gallstones are formed when the normal balance of bile acids and phospholipids that are pumped from the liver into the gallbladder is disrupted. If bile becomes supersaturated with cholesterol, some of the cholesterol precipitates out as crystals, which, under conditions created by the chemical imbalance, can form gallstones. These entities then

trigger inflammation, which is the major symptom of patients with CGD.

In their studies, Mangelsdorf, Moschetta, and Bookout sought to determine the role of a protein known as farnesoid X receptor (FXR), which controls genes whose proteins regulate the transport of the liver's bile acids and phospholipids. The researchers were prompted by the results of previous studies, which had indicated that FXR's level of activity is low in strains of mice susceptible to gallstone disease.

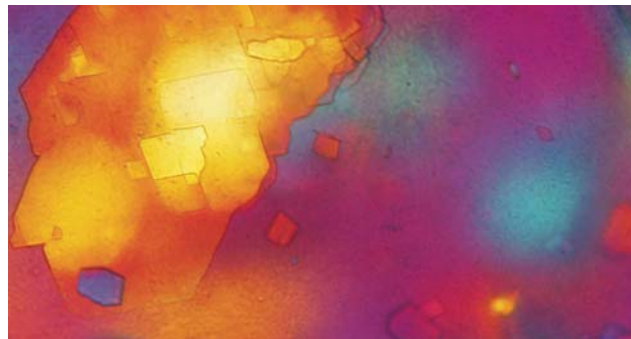
Mangelsdorf says mice are good models for CGD because they have the same genetic regulatory pathways to control the components of bile as humans. Also, the mouse version of CGD physiologically mimics the disease in humans.

The Mangelsdorf team used knockout mice that lacked the *FXR* gene and fed them a "lithogenic" diet (high in cholesterol and other components of bile) designed to induce gallstone formation.

The knockout mice subsequently exhibited cholesterol saturation and lower levels of biliary lipids, resulting in cholesterol crystals—conditions that closely matched those seen in humans with CGD. The researchers also found that the bile acids created the same hydrophobic (water repellent) conditions and inflammation that are hallmarks of the disease in humans. And when they measured the activity of genes known to be involved in transporting lipid components of bile, they found low activity.

"Once we had established that the FXR-deficient animals were much more susceptible than normal animals to getting all the sequelae of CGD, we decided to explore the effects of enhancing FXR activity in a strain of mouse that was known to have FXR but that was also susceptible to the disease," says Mangelsdorf. "We wanted to determine whether a drug could reestablish the proper equilibrium of the bile components."

Thus, in addition to feeding CGD-susceptible mice a lithogenic diet, the researchers also gave them a synthetic compound (code-named GW4064 and owned by GlaxoSmithKline) known to mimic the natural chemical that switches on FXR. The compound's effects on the mice were dramatic, says Mangelsdorf. "Their cholesterol saturation, bile lipids, and bile hydrophobicity were normal. And they showed no cholesterol-crystal precipitation or



David Mangelsdorf, left, found a way to block cholesterol gallstone disease in mice. Polarizing-light microscopy of gallbladder bile shows the deposition of cholesterol crystals, which can form gallstones under the right biochemical conditions.

inflammation.” By contrast, susceptible mice that did not receive GW4064 showed evidence of gallstone formation.

“While we have not shown in this study that the drug that activates FXR cures the disease once it starts, it does prevent gallstones from occurring,” says Mangelsdorf. Although further studies will be needed to determine whether the FXR-activating drug can dissolve gallstones that have already formed, the team’s findings have clinical implications for the prevention and diagnosis of CGD, he says. “The lack of FXR might well be a diagnostic marker for genetic predisposition to CGD.”

Also promising, he says, is the potential for such a drug to prevent pancreatic inflammation and “microlithiasis” in people whose gallbladders have been removed because of gallstones. In this disorder, a sludge of cholesterol-supersaturated bile inflames the bile duct. By restoring the normal properties of bile, the drug would render it less viscous and inflammatory.

Meanwhile, although the synthetic compound used in the research was an expensive experimental substance, says Mangelsdorf, “I have no doubt that the pharmaceutical industry will use these findings as a basis for commercial drug development, provided there are no serious side effects in humans.”

ALZHEIMER’S DISEASE: TROJAN-HORSE THERAPY

The plaques, or amyloid protein chains, that clog the brains of people with Alzheimer’s disease are built up from individual units called β -amyloid (A β) peptides. Pharmaceutical companies have tried to develop effective A β peptide inhibitors, so far without success.

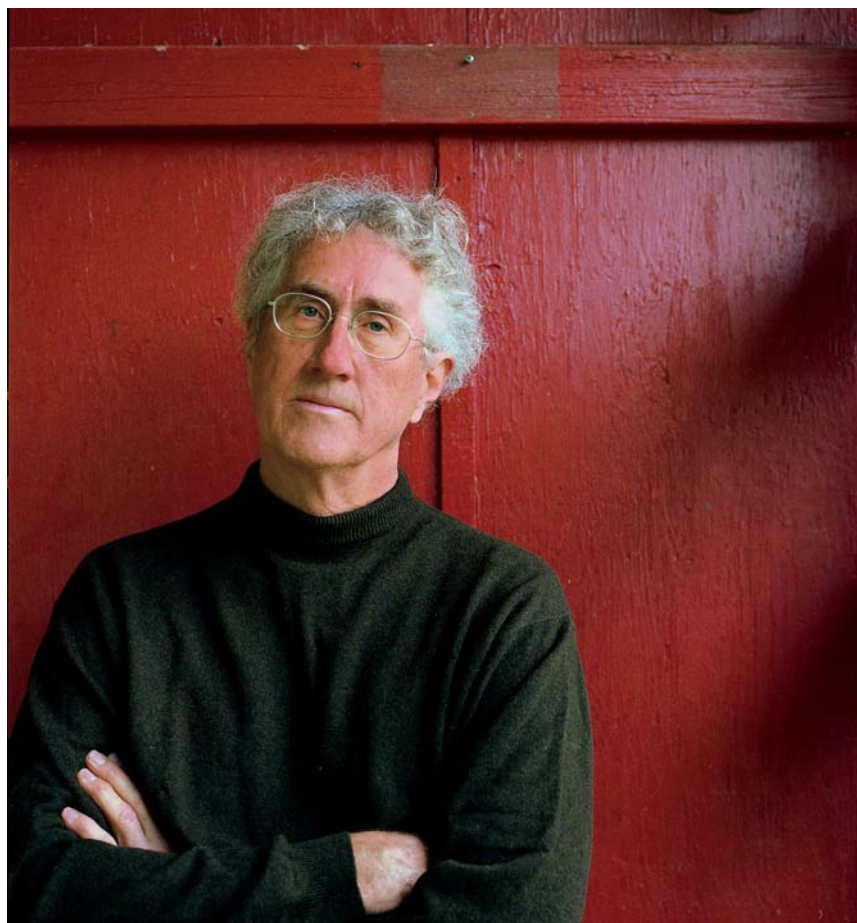
But a new approach pioneered by HHMI researchers protects brain cells in culture by dispatching a small molecule into the cell to enlist the aid of a larger “chaperone” protein, which blocks accumulation of the brain-clogging peptides. This “Trojan horse” technique overcomes a major challenge in drug design—the limited ability of molecules small enough to enter a cell to interfere with interactions between much larger proteins. The researchers say it also might be possible to use the approach to sabotage proteins central to pathogenic organisms, such as human immunodeficiency virus (HIV), and they hope it will be useful for producing drugs to rapidly mutate targets of cancer chemotherapeutics.

HHMI investigator Gerald R. Crabtree and his colleagues Jason E. Gestwicki and Isabella

A. Graef at Stanford University School of Medicine reported their findings in the October 29, 2004, issue of the journal *Science*.

“The insurmountable problem [thus far] has been that protein interactions represent the binding of two large, perfectly matched surfaces, and small-molecule drugs are only a tiny fraction of the size of those surfaces,” says

interaction was to be blocked, and the second site would bind to another, comparably sized protein called a chaperone. Such proteins are ubiquitous in cells and normally serve as “helper” molecules that guide proteins to their proper functional locations. Chaperone molecules are so plentiful in the cell, in fact, that recruiting a fraction of them for use in a treat-



Gerald Crabtree: pairing a small molecule with a large one to make a whole greater than the sum of its parts.

Crabtree. “So even if small molecules are constructed to bind selectively at a site between two such proteins, they either squirt out or the plastic surfaces of the proteins just bend to accommodate them.”

In early experiments, Roger Briesewitz, a former member of the Crabtree laboratory and HHMI fellow who is now on the faculty of Ohio State University, developed the Trojan-horse approach with Crabtree to interfere with protein-protein interactions by designing small molecules with two binding sites. The first site would selectively bind to the protein whose

ment approach would not compromise their normal function, notes Crabtree.

It was Graef’s insight, Crabtree says, that the Trojan-horse technique might be ideal for stopping the formation of toxic amyloid aggregates and thereby preventing Alzheimer’s disease. “Isabella suggested that we try A β peptide as a target because it is small enough that a bulky chaperone protein could possibly interfere with [its] amyloid formation.”

Responding to this idea, Gestwicki constructed a series of small “linker” molecules that would attach to a class of molecules called

FKBP—a family of chaperone proteins found naturally at high concentrations in the cell. The other end of the linker attached to a dye molecule called Congo red, which selectively stains amyloid in muscle and nerves.

In test-tube studies, the researchers found that their Trojan-horse molecules did block the growth of amyloid aggregates from their A β peptide components. In particular, they found that the molecules inhibited growth of the shorter amyloid chains, which are believed to be more toxic to neurons. They also found that, by varying the linker molecules, they could optimize certain pharmaceutical properties of the Trojan-horse assemblage—regarding, for example, its ability to penetrate the cell membrane to enter the cell.

A second round of optimization with their linkers enabled the scientists to achieve even better results. “In fact,” says Crabtree, “we achieved much better protective effects at low concentrations than have been achieved by pharmaceutical companies and by other academic groups using other approaches to inhibiting A β aggregation.”

The next step will be to test the Trojan-horse molecules on mouse models of Alzheimer’s disease and determine whether they impede disease progression.

Crabtree says that if it is successful in these tests, the Trojan-horse approach ultimately might complement other therapies now being tested for Alzheimer’s disease, including anti-inflammatory treatments to prevent neuronal cell death from toxic aggregates and inhibitors of aberrant molecular signaling pathways in Alzheimer’s disease.

Crabtree also speculates that his group’s approach could be applied widely. For example, it might be used to interfere with other clinically important protein-protein interactions, such as those involving enzymes critical to the replication of HIV.

“HIV proteins are difficult drug targets because they can mutate rapidly to render small-molecule inhibitors inefficient,” he says. “Such drugs typically bind only to a few amino acids in the protein, which the virus can easily alter by mutation. But in our approach, we could distribute the binding over a large protein-protein interaction surface, which would be far more difficult for the virus to block. A similar approach could also be taken with rapidly mutating targets of cancer chemotherapeutics.”

—DENNIS MEREDITH



The germination of Yishi Jin’s recent research on nerve regeneration came in a serendipitous social hour.

Nerve Verve

*Bridging physics and biology, researchers use laser-assisted nanosurgery to explore nerve regeneration in the roundworm *Caenorhabditis elegans*.*

When several scientists from Turkey got together in northern California for Thanksgiving 2003, they shared more than just an American tradition and a tasty dinner. Hulusi Cinar, his wife Nese, and Mehmet Fatih Yanik talked about how they might collaborate on an interesting experiment. Yanik, based at Stanford University’s department of applied physics, was building a femtosecond laser nanosurgery system—which shoots pulses of intense laser light that can cut or vaporize a structure precisely within a few hundred nanometers—and he wanted to test it on organisms. The Cinars, both biologists at the Universi-

ty of California, Santa Cruz (UCSC), had been studying the roundworm *Caenorhabditis elegans*, and they were intrigued by the prospect of observing behavioral effects in the animal that resulted from precisely cut nerves.

Eager to proceed with the experiment—and help bridge the fields of physics and biology along the way—the three enlisted the aid of UCSC biologists Yishi Jin and Andrew D. Chisholm as well as engineering physicist Adela Ben-Yakar (then at Stanford, now at the University of Texas at Austin). By the following Thanksgiving, the group had obtained its experimental results, since published in the December 16, 2004, issue of *Nature*. “This is why it’s good to have social hours,” says Jin, an HHMI investigator. “Although many times ideas are not followed up, this time they were.”

Best of all, the team developed a new model for studying nerve regeneration that might shed light on how to treat neurodegenerative diseases, nerve damage, and spinal-cord injury.

The two groups, it turned out, made for an excellent collaboration. The Santa Cruz