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RESEARCH NEWS

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Researchers Identify Hundreds of Human Genes that Impact West Nile Infection

The West Nile virus is a lean genetic machine. It contains just enough genetic material to build ten proteins by itself, and relies on its hosts' cellular machinery to build everything else it needs.

Now Howard Hughes Medical Institute investigator Erol Fikrig and his colleagues have identified more than 300 human genes that influence West Nile virus infection. More than 20 of these cooperate with the virus, whereas the others help cells resist it. Fikrig's group also tested the related dengue fever virus in search of genes important to both viruses. Their results are reported in an August 6, 2008, advance online publication of the journal *Nature*.

> "We wanted to ask the question in the global sense: What is the genetic dictionary of the West Nile virus?"
> **— Erol Fikrig**

The finding hints at common weaknesses that could be used to target flaviviruses: the class of viruses that includes West Nile and dengue fever, as well as yellow fever, encephalitis, and hemorrhagic disease. Some of the genes hit the same pathways, Fikrig says, and disabling those pathways might stop flaviviral infections. The process could be as simple as targeting one of the genes the team identified in its study. You don't need to destroy the whole bridge to stop a car going across it, he says.

Fikrig's team created 21,000 experimental cell lines, each missing a different human gene, using a technique known as RNA interference. We wanted to ask the question in the global sense: What is the genetic dictionary of the West Nile virus? Fikrig says.

The team exposed the cell lines to a fluorescent version of the West Nile virus for 24 hours, and then inspected them under a microscope to measure the virus's spread. The nice thing with West Nile virus is you can do immunofluorescence to get a rough but reasonable estimate of how much

virus is in the cell, Fikrig says. In this way, they could identify which cells had a lower level of viral infection.

For the most part, the RNA screens in the past have been done with fruit flies, Fikrig says. We wanted to go as close to human cells as possible. The team used a common human cell line known as HeLa cells, derived from a cervical cancer patient who died in 1951. The cells are able to live outside the human body and are often used as an experimental model.

The measurements were painstaking, because it is easy to get false results, and the screens had to be repeated multiple times to ensure accuracy. Otherwise, it's garbage in; garbage out, Fikrig says.

In 283 of the cell lines, the virus grew at least twice as fast as it did in unaltered cells, indicating that those missing genes help human cells resist the West Nile virus. All but 10 of those genes had never before been associated with this role. Another 22 altered cell lines resisted infection better than their counterparts, demonstrating that those 22 genes facilitate infection. Only one of those genes had previously been known to play a role in West Nile infection.

Knowing which genes promote or repress the virus is useful, Fikrig says. Genes that promote susceptibility can be used to learn how the virus enters the cell or replicates. At the same time, it might be possible to enhance the natural resistance conferred by the other set of genes.

The team then tested the dengue fever virus on cell lines with each of the 305 human genes that influence the West Nile virus. About 40 or 50 of the 300 were common between the two experiments. We hope there are others in common with several—if not the majority of—flaviviruses, Fikrig says. Such common pathways could be useful for developing drugs against the entire class of viruses.

Now that the team has the dictionary for West Nile virus, they are testing Japanese encephalitis and other flaviviruses in the same way as the dengue fever virus to look for more overlapping genes.

While these studies were only in vitro, the next step is to test these genes in mice. Our goal is to develop therapeutics for all flaviviruses, Fikrig says. But what you get in a HeLa cell in vitro might be very different from what you find in vivo. We're cautiously optimistic.