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## New Research Reveals How *Giardia* Parasite Changes Its Appearance

Like a gang of bandits that changes clothes after a heist to avoid capture, the intestinal parasite *Giardia lamblia* alters its appearance to outwit the human immune system. Now, after a 20-year search, experiments by Howard Hughes Medical Institute international research scholar Hugo D. Luján reveal how the parasite shifts disguises.

The findings, published in the December 11, 2008, issue of the journal *Nature*, are a first step toward developing vaccines against *Giardia* and other pathogens that change their outer coats as a defense strategy, said Luján, a scientist at the Catholic University of Córdoba in Argentina. Currently, there is no vaccine to protect against *Giardia* infection, which is a major cause of diarrhea in much of the world. The World Health Organization estimates that more than 200 million people contract *Giardia* each year. The parasite, which is spread through feces in contaminated soil or water, can persist as a microscopic cyst for months or years. *Giardia* infections can also last for months, as the parasite has evolved a cunning defense to evade the human immune system.

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— Hugo D. Luján

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Luján has been trying to understand the parasite's defense system since the 1990s, when he worked in Theodore Nash's laboratory at the National Institutes of Health. In 1998, Nash discovered that *Giardia* shifts key molecules on its surface, through a process called antigenic variation. Just as the human immune system learns to identify and attack *Giardia*, the parasite displays a new molecule, or antigen, on its surface, Nash found. This switching confuses the immune system and makes the parasite invisible to it.

In describing *Giardia*'s cunning deception, Luján likens the parasites to a gang of bandits that can switch their jackets (surface antigens) at will. Imagine someone calls the police and says, 'You have to catch that bunch of guys with blue jackets.' So the police go there, but the robbers have changed to green jackets, Luján said. The police don't know what to do. It is something the 'bandits' do to evade capture by the police.

Since Nash's discovery, *Giardia* researchers have been trying to understand how the parasite switches coats. The *Giardia* genome contains nearly 200 genes that code for surface antigens. Despite the large number of genes, each parasite displays only a single antigen derived from a single gene on its surface at any given time. Normally, the RNA instructions for each gene are copied from the parasite's DNA then run through the cell's internal machinery to create proteins, including surface antigens. As Luján and his group delved into this mystery, he discovered that the molecular machinery in the parasite actually reads and transcribes most of the 200 surface antigen genes, but only one antigenic protein makes it to the outer coat on the parasite's surface. That observation suggested to Luján that the RNA instructions called messenger RNA had been created and were inside the organism, but something was stopping the RNA from being translated into proteins and expressed on the cell's surface. That was a surprise, Luján said. Because it takes a lot of energy for the organism to transcribe all of those genes into messenger RNA.

So Luján sought to find out why most of the parasite's RNA never gets translated into a working surface antigen. Something must be interfering with that process for *Giardia*'s surface antigens. He suspected that a powerful cellular mechanism called RNA interference, or RNAi, might be the cause of the holdup. Discovered a decade ago, RNAi identifies short segments of RNA and then destroys all RNA that matches the segments. The result: None of the protein that would have been created by that RNA is released into the cell. Researchers originally found that RNAi is a protective mechanism that helps cells fight off invading viruses. But as the new work by Luján shows, the process can also control which genes are switched on and off in *Giardia*.

In a series of experiments, Luján found that the cellular machinery involved in RNAi squelches most of the RNA in *Giardia* that codes for surface antigens. At any given time, in fact, only the RNA for a single type of antigen is left untouched. The rest is destroyed. At that point, we realized RNAi was strongly linked to antigenic variation, Luján said.

Next, Luján inhibited the enzymes involved in RNAi in *Giardia*. Again, he made a surprising discovery: The cells with defective RNAi machinery displayed many surface antigens simultaneously a kind of parasitic Technicolor dream coat. That was the direct link between enzymes involved in the RNAi pathway and antigenic variation in *Giardia* that we had been looking for, Luján said.

Now Luján is working on a series of experiments to find out whether genetically altered strains of *Giardia* that display many surface antigens could be used to make a vaccine that would help the immune system identify

the parasite's many disguises. If it works, it would be proof of principle that abolishing antigenic variation in *Giardia* by knocking down RNAi (could) have a major impact in developing vaccines against microorganisms that undergo antigenic variation, Luján said.