New images may improve vaccine design for deadly rotavirus

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Howard Hughes Medical Institute researchers are reporting the first detailed molecular snapshots of a deadly gastrointestinal virus as it is caught in the grasp of an immune system molecule with the capacity to destroy it. The images could help scientists design a more effective vaccine against rotavirus, a lethal infection that kills more than 500,000 children worldwide each year. The discovery is timely.

Last week the World Health Organization recommended that rotavirus vaccination be included in all national immunization programs worldwide. Virtually every child in the world becomes infected with rotaviruses before developing natural immunity. But each year an estimated two million children are hospitalized because rotavirus infection results in severe dehydration caused by diarrhea and vomiting.

Both natural and vaccine-induced immunity occur only after the immune system has "seen" the virus and generates neutralizing antibodies. These soldiers of the immune system seek out and attach to rotavirus particles, rendering them unable to infect cells.

In the new experiments, Howard Hughes Medical Institute (HHMI) researchers have mapped the structure of an antiviral antibody clamped onto a protein called VP7 that stipples the surface of rotavirus. The structural map reveals intimate new details about how the antibody interferes with VP7, a protein that helps the virus infect cells. The information may be useful in designing a new generation of rotavirus vaccines that could be easier to store and administer than current vaccines, said the researchers.

HHMI investigator Stephen C. Harrison and colleagues at Children's Hospital Boston and Harvard University published their findings in the June 12, 2009, issue of the journal *Science*.

Rotaviruses replicate mainly in the gut, where they infect cells in the small intestine. The virus has a triple-layered protein coat, which allows it to resist being chewed up by digestive enzymes or the gut's acidic environment. Rotavirus does not have an envelope covering its protein shell. A virus' envelope helps it enter host cells, and viruses without envelopes face significant hurdles in penetrating the membrane of the cells they infect. "Since they have no membrane of their own, they must therefore perforate a cellular membrane to gain access to the cytoplasm (the interior of the cell)," he said.

The new research shows that as rotavirus matures inside an infected cell, it assembles a kind of "armor" coating made principally of VP7 and a "spike" protein called VP4. When the mature virus particle exits one cell to infect a new cell, it perforates the endosomal membrane of the target cell by thrusting in its VP4 spike like a grappling hook.

The virus' ability to infect cells depends on a critical structural change that quickly removes the coat from the interconnected VP7 proteins -- an event that unleashes the spike protein. Although researchers still do not know precisely what triggers the uncoating of VP7, they do know that it appears to happen when the virus senses a lowered concentration of calcium in its environment.

"VP7 sort of closes over VP4 locking it in place like the metal grills that surround a tree planted on a city sidewalk," explained Harrison. "And it is the loss of VP7 in the uncoating step that triggers VP4 to carry out its task."

To get a closer look at how antibodies latch onto VP7 and neutralize the virus, Harrison and his colleagues used x-ray crystallography to examine the molecular architecture of VP7 in the grasp of a fragment of the antibody. X-ray crystallography is a powerful tool for "seeing" the orientation of atoms

and the distances separating them within the molecules.

Before Harrison's team could use x-ray crystallography, however, they first had to crystallize VP7 in complex with the antibody fragment. Only after that step was completed, could they move on to bombarding those crystallized proteins with x-rays. Computers helped capture the diffraction patterns that emerged as the x-rays scattered from the crystal lattice. By rotating the crystallized protein complexes through multiple exposures, the researchers could record enough data to calculate three-dimensional models, which exposed the underlying architecture of VP7 and the antibody fragment.

The resulting detailed structural map of the VP7-antibody protein complex revealed that the antibody neutralizes the virus by preventing the VP7 proteins from dissociating, said Harrison. "Normally, calcium creates a bridge between VP7 molecules that holds them in place until uncoating," he said. "Our structure revealed that the antibody makes an additional bridge, cementing the subunits together, making the virus resistant to the uncoating trigger and preventing it from infecting cells."

Current rotavirus vaccines consist of weakened live virus that triggers the immune system to produce neutralizing antibodies. However, the new structural findings suggest how researchers might engineer a different type of rotavirus vaccine consisting only of immune-triggering protein, said Harrison. This protein-only vaccine could be made of a chemically linked complex of VP7 molecules that would stimulate the immune system more vigorously to produce anti-rotavirus antibodies.

While live-virus-based vaccines have been effective, said Harrison, they have drawbacks that a protein-based vaccine might overcome. The virus-based vaccines are perishable and require refrigeration, but vaccines based on proteins are more stable and can be stored at room temperature. Another benefit, said Harrison, is that protein-based vaccines could be combined with other protein vaccines in a "cocktail" that would cut down on the number of clinic visits since blending cannot be done so readily with virus-based vaccines. These advantages could make protein vaccines especially useful in developing countries that lack an extensive public health infrastructure and where the vast majority of childhood deaths from rotavirus occur, Harrison said.

Harrison's interest in the structure of viruses is wide ranging, and over the years, his laboratory has made numerous seminal discoveries showing how viruses assemble and enter cells. Rotavirus has been a project of intense interest for Harrison and fellow HHMI investigator Nikolaus Grigorieff at Brandeis University, who have collaborated to develop innovative techniques to visualize important structural features of rotavirus. In a recent article published in the Proceedings of the National Academy of Sciences, Grigorieff and Harrison have applied cryo-electron microscopy (cryo-EM) and single particle reconstruction techniques to visualize key structures that rotavirus uses to infect cells. Cryo-EM is one of the few techniques capable of visualizing large, dynamic molecules.

In preparing for cryo-EM, researchers first immerse the virus particles in water solution and then abruptly freeze them in supercold liquid ethane. The rapid freezing imprisons the bound complexes in ice, thus preserving the particles' native structure. Using an electron microscope with a highly collimated beam (whose rays are nearly parallel) to avoid damaging the molecules, the scientists obtained images of thousands of captive particles. The scientists then employed sophisticated computerized image analysis to align thousands of cryo-EM images, average them, and obtain one clear image from all of them. These advances helped the researchers avoid the time-consuming and painstaking task of making crystals, as well as the need for radiation, which sometimes damages the structures.

"This is a major advance in cryo-EM," says Harrison. "It has brought cryo-EM to near-atomic resolution, just like crystallography. This technique allows us to get structural information so much more rapidly about states of the structure that you could never visualize before."

Source: Howard Hughes Medical Institute (news : web)

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