Study finds a weak spot on deadly ebolavirus

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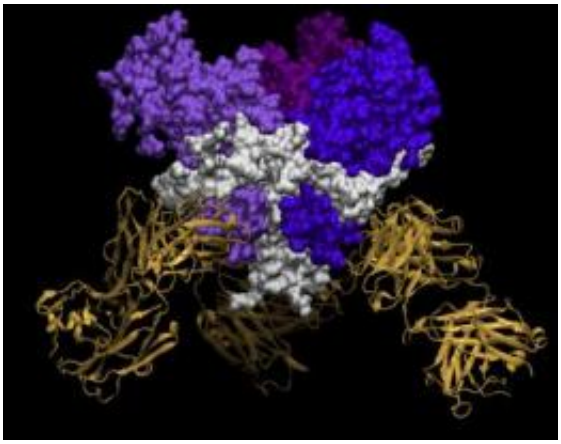

The new research shows how an antibody neutralizes the deadly Sudan virus. Credit: Photo Courtesy of the Scripps Research Institute

Scientists from The Scripps Research Institute and the US Army's Medical Research Institute of Infectious Diseases have isolated and analyzed an antibody that neutralizes Sudan virus, a major species of ebolavirus and one of the most dangerous human pathogens.

"We suspect that we've found a key spot for neutralizing ebolaviruses," said Scripps Research Associate Professor Erica Ollmann Saphire, who led the study with US Army virologist John M. Dye.

The new findings, which were reported November 20, 2011, in an advance online edition of *Nature Structural & Molecular Biology*, show the antibody attaches to Sudan virus in a way that links two segments of

its coat protein, reducing their freedom of movement and severely hindering the virus's ability to infect cells. The protein-linking strategy appears to be the same as that used by a previously discovered neutralizing antibody against the best-known ebolavirus species, Ebola-Zaire. The new study suggests that this may be the best way for vaccines and antibody-based therapies to stop ebolaviruses.

Deadly Outbreaks

Ebolaviruses first drew the attention of the medical world with simultaneous deadly outbreaks in 1976 in the nations of Sudan and Zaire (currently known as the Democratic Republic of the Congo). These two outbreaks were caused by the two major viruses: Ebola-Sudan and Ebola-Zaire, and early field studies showed that sera from patients that survived one virus could not help patients infected with the other. . Both viruses persist in animal hosts–probably bats–and when they spread to humans, typically cause severe hemorrhagic fevers, killing up to 90 percent of people they sicken. Although not as contagious as influenza or measles, ebolaviruses can be transmitted in bodily fluids including exhaled airborne droplets, and scientists who study these viruses are generally required to use special "Biosafety Level 4" facilities. The US government regards the ebolaviruses as a potential bioterror threat.

Ebolavirus researchers hope to develop a vaccine that could be used to protect health workers and others in the vicinity of ebolavirus outbreaks, as well as an antibody-based immunotherapy that could help infected people survive. However, these tasks are complicated by the fact that there are now five recognized species of ebolavirus: Ebola-Zaire, also known simply as Ebola virus; Taï Forest virus; Reston virus; Bundibugyo virus; and Sudan virus.

"These species differ enough from each other that neutralizing antibodies to one don't protect against the rest," said Ollmann Saphire.

"Sudan virus is a particular concern because it has caused about half of the ebolavirus outbreaks so far, including the largest outbreak yet recorded."

Uncovering the Body's Natural Protection

US government researchers recently demonstrated that an experimental vaccine containing proteins from Ebola and Sudan viruses provides monkeys with some protection against those viruses. But precisely how the vaccine works is unclear, and it has never been tested in humans. Moreover, until now no laboratory has isolated a neutralizing antibody against Sudan virus.

To find such an antibody, Dye and his colleagues at Fort Detrick, Maryland, injected lab mice with a harmless virus engineered to make copies of the Sudan virus coat protein. The coat protein provoked the mice's immune B cells to make various antibodies against it, and the scientists were able to reproduce the mice's repertoire of antibodies by harvesting their B-cells and culturing them in the lab. Testing each type of antibody for its ability to block the infection of cells with Sudan virus, the researchers found one good candidate, antibody 16F6, which not only neutralized Sudan virus in the lab dish but also significantly delayed the deaths of infected mice. They then sent 16F6 to Ollmann Saphire's lab at Scripps Research in California.

"We were very excited about developing this antibody as a potential treatment for Ebola virus," said Dye. "Collaborating with the Ollmann Saphire lab to determine the binding site was the perfect complement to our previous work"

Ollmann Saphire's lab specializes in the use of X-ray crystallography and related techniques to visualize the atomic-scale details of viruses bound by antibodies. These details reveal where on a virus an antibody binds,

and if the antibody is one that neutralizes a virus's ability to infect cells, its binding site usually offers important clues to the virus's workings and vulnerabilities.

In the new study, Ollmann Saphire's team found that 16F6 attaches to the Sudan virus in a way that links two segments of the viral coat protein. The virus is known to use one of these segments, GP1, to grab hold of a host cell. When this happens, the cell automatically brings the virus inside, encapsulated within a bubble-like chamber known as an endosome. Normally the cell would destroy the contents of such an endosome, but Sudan virus–like some other viruses–employs its other viral coat-protein segment, GP2, to fuse to the wall of the endosome so that it and the rest of the virus escape into the doomed cell's interior. Antibody 16F6 seems to prevent this fusion process from happening by keeping GP2 bound to GP1.

"The virus is like a wolf in sheep's clothing because its outer part is covered with human sugar molecules, that the antibodies do not see as foreign," said Ollmann Saphire. "The binding site of the 16F6 antibody is one of the few places where viral protein is exposed, and it's exposed because it's a place where GP1 and GP2 need to be free to move." To fuse to the endosomal wall, GP2 must separate from GP1 and uncoil itself. When it is held fast to GP1 by the antibody 16F6, GP2 can't uncoil and perform its function–and so the Sudan virus, instead of escaping into the relatively unprotected interior of the cell, stays within the endosome and is eventually destroyed.

A Strategy Against Ebolaviruses

Ollmann Saphire and her colleagues suspect that 16F6's protein-linking strategy is the best one that antibodies have against ebolaviruses. The antibody's binding site on the Sudan virus coat protein is virtually the same as the binding site of an Ebola-Zaire-neutralizing antibody known

as KZ52, which Ollmann Saphire and Scripps Research colleague Professor Dennis Burton found and analyzed three years ago. KZ52 is derived from antibodies made by an African patient who survived an Ebola-Zaire outbreak in 1995, and aside from 16F6 it is the only ebolavirus-neutralizing antibody whose binding site has been determined with X-ray crystallography.

"We think it's not just a coincidence that these two different antibodies, evoked in two different host species by two different ebolaviruses, use the same strategy of linking GP1 and GP2," Ollmann Saphire said.

She and her colleagues now are trying to obtain structural data on several other ebolavirus-neutralizing antibodies, and she suspects that at least one of these also works by linking GP1 to GP2. "There may be other neutralizing sites on ebolaviruses, but so far the only one we've found is this one," she said.

The recognition that ebolavirus-neutralizing antibodies share this protein-linking strategy should guide the further development of vaccines and immunotherapies. "It helps us to understand more precisely what an ebolavirus vaccine or immunotherapy ought to do," Ollmann Saphire said.

More information: "A shared structural solution for neutralizing ebolaviruses," *Nature Structural & Molecular Biology* (2011).

Provided by The Scripps Research Institute