Discovery could lead to better control of hemorrhagic fever viruses

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Researchers report discovering the receptor through which a group of life-threatening hemorrhagic fever viruses enter and attack the body's cells, and show that infection can be inhibited by blocking this receptor. The findings, to be published online by the journal *Nature* on February 7, give a clue to the high lethality of New World arenaviruses, suggest a way of reducing the severity of infection, and point the way toward a sorely needed treatment strategy.

The four viruses, known as the Machupo, Guanarito, Junin and Sabia viruses, cause Bolivian, Venezuelan, Argentine and Brazilian hemorrhagic fever, respectively, with mortality rates of about 30 percent. No vaccine is available, though a weakened form of Junin virus has been given to Argentinean farmers with some success. In addition to causing occasional disease outbreaks, mostly in poor, rural areas of South America, the viruses are of U.S. government interest because of their potential as bioterrorism agents. All four are classified as NIAID Category A Priority Pathogens and must be handled in Biosafety Level 4 containment facilities.

The researchers, led by Hyeryun Choe, PhD, of Children's Hospital Boston's Pulmonary Division, and Michael Farzan, PhD, of Harvard Medical School (HMS), first investigated the Machupo virus. To identify its cellular receptor, they made copies of the "spike" protein, used by the virus to gain entry into cells, and added it to cells from African green monkeys, known to be highly susceptible to Machupo virus infection. Later, they broke the cells open and isolated the spike protein and the

cellular protein to which it had attached itself. Then, using a technique called mass spectrometry, they analyzed this attached cellular protein to determine its identity.

The receptor, identified in Choe's lab by Jonathan Abraham, PhD, an MD-PhD student at HMS, turned out to be transferrin receptor 1 (TfR1), a well-known protein that is key in enabling cells to take up iron. Additional studies, performed in Farzan's lab by HMS graduate student Sheli Radoshitzky, confirmed that TfR1 is also the receptor for the other three New World arenaviruses. (Abraham and Radoshitzky are both first authors on the study.) Expertise from Nancy Andrews, MD, PhD, an expert in iron metabolism at Children's, sped up the work.

Although not all hemorrhagic fever viruses use TfR1 to enter the body's cells, the discovery may help explain why these viruses wreak such havoc, damaging multiple organs and causing bleeding under the skin, in internal organs, and from orifices like the mouth, eyes or ears.

Because of TfR1's essential function in transporting iron into cells, it is found on the surface of virtually every cell of the body. It is abundant on endothelial cells, which line blood vessels, a fact that may help account for the bleeding and organ damage caused by the viruses. TfR1 is also especially abundant on activated immune cells – the very cells that mobilize to fight the viruses – making them especially vulnerable to infection.

"This may help explain why mortality is so high," says Choe, the study's senior author.

Choe now hopes to translate these findings into treatments to contain natural or intentional outbreaks of New World hemorrhagic fever. Serendipitously, several anti-TfR1 antibodies have already been developed as anticancer therapeutics (cancer cells are also high in TfR1),

and some have already been through clinical trials. Choe's lab will test these antibodies, hoping to find one that inhibits virus entry without compromising TfR1's essential function in cellular iron uptake.

"If some of these antibodies work, they could be used clinically fairly soon," Choe says.

Coincidentally, Stephen Harrison, PhD, a structural biologist and Howard Hughes Medical Institute investigator at Children's, had crystallized TfR1 and determined its 3-dimensional structure in 1999. Knowledge of TfR1's structure will speed up the Choe lab's efforts to pinpoint the parts of the molecule that are exploited by New World hemorrhagic fever viruses, which is necessary for the development of targeted antiviral drugs that block those parts, but not the parts involved in iron uptake.

The findings of Choe and colleagues also suggest that iron supplements may reduce the severity of New World virus infections. Past studies have shown that when the iron level in the body is low, the number of transferrin receptors in tissues increases. Consistent with these findings, Choe's team found that New World arenaviruses infect cells more efficiently when iron levels are low, and that adding iron to cultured cells makes them less susceptible to infection. Choe notes that New World hemorrhagic fever outbreaks mostly occur in poor rural areas, where people are often deficient in micronutrients, including iron, possibly predisposing them to more severe infection when exposed to the rodents that carry the viruses.

Choe's lab is now trying to find the cellular receptor for other viruses that cause hemorrhagic fever in humans. In 2003, Choe's lab collaborated with Farzan's lab to identify angiotensin converting enzyme2 (ACE2) as the receptor for the SARS virus.

Source: Children's Hospital Boston