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Ebola's Deadly Secrets

Researchers Identify Two Main Targets for the Virus; Progress Made in Developing Vaccine

A team of HHMI researchers has found a possible clue to how the Ebola virus consumes the human body so quickly.

In studies published in today's issue of the journal *Science*, Gary Nabel, a former Hughes investigator at the University of Michigan, and colleagues report that they have identified two of the virus's main targets in humans: neutrophils, which are white blood cells that contribute to the inflammatory defense system, and endothelial cells, which line the blood vessels.

"We're far from understanding in molecular detail why this virus is so deadly," said Nabel, "but we now know at least two of the virus's primary targets in the human body."

Ebola virus contains seven genes that encode eight proteins. Two of the proteins, a transmembrane glycoprotein (GP) that is expressed on the surface of the virus, and a secreted form of GP (sGP) are produced from a single gene. Researchers have suspected that GP and sGP are involved in Ebola's ability to evade the immune system and cause hemorrhagic fever. Despite their suspicions, scientists have had little idea of what GP and sGP are actually doing during the course of infection.

Once Nabel was convinced that his laboratory could safely handle work on these proteins, his team set about the task of figuring out the function of GP and sGP. They began by inserting DNA encoding the proteins into human cells and asked which cell types these "transfected" Ebola glycoproteins adhered to. Nabel says he was surprised when his team found that sGP bound to neutrophils through a specialized receptor found on those cells. In contrast, GP-transfected cells preferentially attached to endothelial cells. This was determined by putting Ebola GP onto the surface of a mouse retroviral vector which carried a reporter gene to detect infection.

"It is possible that sGP interferes with early stages of a protective inflammatory response," Nabel said, "and GP's targeting endothelial cells may set the stage for the hemorrhagic symptoms of the disease."

Last month, Nabel's team announced that it had produced a vaccine against the Ebola virus that primes the immune system to ward off the deadly effects of Ebola infection.

If the Ebola vaccine continues to perform well in upcoming primate studies, it might soon be tested experimentally in lab workers exposed to the lethal microbe, Nabel says. The vaccine may eventually be used to protect residents in areas susceptible to outbreaks of the fatal infection, which kills its victims by causing internal and external bleeding, loss of liver and kidney function, vomiting and diarrhea. A variation of the vaccine might offer protection against Ebola's deadly cousin, Marburg virus, one of four known RNA filoviruses.

"I'm not taking it for granted that the vaccine will work in primates or humans," Nabel said. "If it doesn't work there are probably things we can do to improve it." The report from Nabel and his team, which includes scientists at the Centers for Disease Control (CDC), appeared in the January 1998 issue of *Nature Medicine*.

"[This] brings us one swing closer to finding a vaccine that will knock this virus out for good," wrote Thomas Folks of the CDC in an accompanying *News & Views* article in *Nature Medicine*. Although the main centers of outbreak of the filoviruses have been central and northeastern Africa, "the threat of these lethal viruses emerging at any moment, anywhere, is real," Folks wrote.

Guided by the principles of genetic immunization, Nabel's team inserted genes from the Ebola virus into plasmid DNA to produce proteins that alert the host's immune system that an assault is underway. Since the proteins used by Nabel's group were harmless, their presence provided an early warning that primes the host to fight the virus if it is encountered.

The researchers included the genes for three Ebola proteins in their vaccine: GP, sGP and a nucleoprotein (NP) that provides internal structure within the virus. The genes from Ebola were inserted into bacterial plasmid DNA which was then injected into the leg muscles of mice. The muscle cells produced Ebola proteins, which elicited an immune response in the mice. Nabel's group found that in mice both proteins could stimulate B cell antibodies but only the glycoprotein could elicit a response from killer T cells.

Moving from mice to guinea pigs, which are as susceptible to Ebola virus as humans, the scientists injected two different groups of guinea pigs with the vaccine. The guinea pigs exposed to Ebola virus two months after vaccination, when antibody production is still high, showed strong immunity

with either GP, sGP or NP immunization. A second group of guinea pigs challenged with Ebola virus four months after immunization did not fare as well. Only the animals vaccinated with the sGP and GP were able to fight off exposure to the virus. "An antibody response alone is not enough to ward off the virus," Nabel said. "You need both antibody and cellular immunity."

Nabel confessed to being momentarily "just bowled over" when the Ebola vaccine worked as hoped. "We made an educated guess that worked out," he said, "and now we have tools that we can use to try to generate immunity in cases where it doesn't naturally occur."