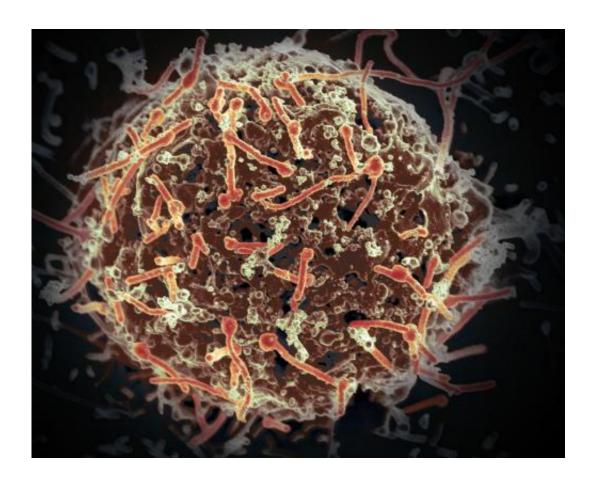# Ebola virus mutations may help it evade drug treatment

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The Ebola virus, isolated in November 2014 from patient blood samples obtained in Mali. The virus was isolated on Vero cells in a BSL-4 suite at Rocky Mountain Laboratories. Credit: NIAID

Genetic mutations called "escape variants" in the deadly Ebola virus appear to block the ability of antibody-based treatments to ward off

infection, according to a team of U.S. Army scientists and collaborators. Their findings, published online this week in the journal *Cell Reports*, have implications for the continued development of therapeutics to treat Ebola virus disease, which has claimed the lives of over 11,000 people in West Africa since last year.

Ebola virus overruns the immune system, thus overwhelming the host's ability to fight off the infection. One strategy for treatment is based on the administration of a "cocktail" of antibodies that have the ability to neutralize the virus and allow the host to mount an effective immune response. One such cocktail, known as MB-003, has demonstrated efficacy in nonhuman primates infected with the virus. MB-003 and ZMAb were early formulations used in proof-of-principle trials that led to the more advanced formulation, ZMapp, currently in development and authorized for compassionate use in the West African Ebola outbreak response.

To understand the reasons for the improvement, investigators at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) Center for Genome Sciences (CGS) examined nonhuman primates that succumbed to Ebola virus despite being treated with MB-003 one or two days post-infection. Viral nucleic acids were isolated from blood samples drawn at multiple time points and were sequenced to provide a description of the viral population. This approach is routinely utilized at USAMRIID to study the development of viral therapeutic resistance against antiviral countermeasures, according to Dr. Gustavo Palacios, director of the center and senior author of the study.

Two clusters of changes in the viral genome were observed in one of the animals that succumbed. Several of those changes corresponded with the viral target sites of two of the cocktail's antibodies, triggering an in-depth molecular analysis of the development and impact of those changes.

"The molecular analysis allowed us to see where the cocktails were inducing changes in the genome, and to link those changes to the treatment failure," said co-first author CPT Jeffrey Kugelman, Ph.D., of USAMRIID. Based on these findings, tissues at selected time-points were used for viral isolation, which finally allowed the "rescue" of the mutated virus.

"When this rescued virus was sequenced, we observed that the clusters of changes had progressed from affecting a small portion of the viral population to becoming mutations—permanent changes in the genome—without disrupting any major viral functions, including the ability to cause infection."

Subsequently, the rescued virus was tested for neutralization against the cocktail and its individual components, demonstrating the inability of the therapeutic to control replication.

"At this point, we knew that the mutations were, in fact, 'escape variants' that were cumulatively responsible for reduced efficacy of the MB-003 therapeutic," Palacios said. "However, we were unsure of the frequency of this type of event."

To help answer that question, the scientists analyzed other independently conducted but similarly designed studies of MB-003.

"We were able to identify, using the same tools, a second animal with a similar pattern of changes," Kugelman noted. "Strikingly, the two animals had four sites in common. This information leads us to believe that these sites are under 'selection pressure' by the therapeutic, meaning that the antibody cocktail binds and promotes elimination of the original virus, while the escape variants continue the infection."

Palacios added that further work is needed to document each individual

mutation's effect on each of the three antibodies.

"Our research has already established that a few amino acid changes may be sufficient to erode the binding of multiple antibodies in a cocktail," he said. "It would be very important to determine which changes have a direct impact on binding."

The team's findings highlight the importance of selecting different target domains for each component of the therapeutic cocktail to minimize the potential for viral escape, according to the authors. Two of the antibodies in the MB-003 cocktail target a related region of the Ebola virus glycoprotein, and thus are more susceptible to localized changes affecting multiple antibodies. Furthermore, these findings are important for scientists studying Ebola virus to provide a basis for how quickly the virus can adapt to therapeutics. This rate will be important for predictive models of therapeutic resistance.

Ebola virus causes severe hemorrhagic fever in humans and nonhuman primates with high mortality rates and continues to emerge in new geographic locations, including West Africa, the site of the largest outbreak to date. Over 28,000 confirmed, probable and suspected cases have been reported in Guinea, Liberia and Sierra Leone, with more than 11,000 reported deaths, according to the World Health Organization.