Ebola Hemorrhagic Fever: Genetic Biomarkers and Vaccine Development

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THE EBOLA VIRUS causes a highly virulent systemic disease that involves viral hemorrhagic fever, affects multiple organ systems, and leads to internal bleeding and, in most cases, death. The World Health Organization estimates that as of August 31, 2014, there have been 3,685 cases and 1,841 deaths during the current outbreak (World Health Organization, 2014). The Centers for Disease Control and Prevention estimates that by September 30, 2015, there will be approximately 8,000 cases in Liberia and Sierra Leone, making the current outbreak the largest in history (Centers for Disease Control and Prevention, 2014).

Given its high fatality rates, reaching 90% of human and nonhuman primates, there is little understanding of the pathogenesis of the Ebola virus. The infection itself works by inhibiting activation of lymphocytes and dysregulating intravascular coagulation (Reed *et al.*, 2004; Geisbert *et al.*, 2003b). Previous research done during the 2000–2001 outbreak in Sudan attempted to shed light on the pathology of the disease, revealing that hemorrhagic symptoms may be caused by elevated cytokine levels and coagulation (McElroy *et al.*, 2014).

Since the first documented outbreak of Ebola in 1976, there have been several experimental treatments with varying degrees of success. More recently, a study found that ZMapp, composed of three humanized monoclonal antibodies (c13C6, c2G4, and c4G7), was successful in treating rhesus monkeys with the infection (Qiu *et al.*, 2014). The treatment was used on three health care volunteers from North America and Europe and was effective in fighting the infection. However, these experimental drugs are in incredibly short supply, which makes the need for new treatments greater. Existing research on the genetic biomarkers of the disease may provide valuable pathways toward disease prevention and direct development of these new treatments.

In March 2014, at the beginning of the current outbreak in Guinea, researchers looked at prior studies showing the positive effect of anticoagulant therapeutics on nonhuman primate test subjects (Garamszegi *et al.*, 2014). This investigation found that rhesus monkeys treated with a recombinant nematode anticoagulant protein c2 (rNAPc2), an inhibitor of tissue factor–initiated blood coagulation, increased the survival rate of the test group from 0% to 33% (Geisbert *et al.*, 2003a). In addition, another previously published study using recombinant human activated protein C (rhAPC) showed that the nonhuman primates responding to

the anticoagulants had lower plasma viremia levels and virus with less virulent proinflammatory and procoagulant properties (Garamszegi *et al.*, 2014).

Garamszegi's research team wanted to determine whether they could identify transcriptional gene signatures that would correlate with survival following infection because the prior studies did not address Ebola virus infection from a transcriptional view.

The team used a test group of 23 rhesus macaques: 19 treated with the anticoagulant therapeutics (11 rhAPC and 8 rNAPc2) and 4 untreated controls. Four of the nonhuman primates infected with a lethal dose of Ebola virus survived (2 treated with rhAPC and 2 treated with rNAPc2).

These results revealed that the treated macaques showed changes in messenger RNA expression. The investigators identified 20 genes that distinguished between the surviving nonhuman primates and the ones that died; these included 16 annotated genes, 13 genetic loci, and 1 microRNA (miRNA).

Several of the genes exhibited early differential regulation before appearance of Ebola symptoms, but more important, several of the identified genes suppress replication in other viruses. The protein ILF3 suppresses the function of viral polymerase, which suggests that the surviving nonhuman primates may have upregulated transcription of these genes to suppress viral replication (Garamszegi *et al.*, 2014).

Given the speed with which the Ebola virus replicates, it may be valuable to explore treatments involving protein ILF3 to suppress the rate of replication.

Another notable finding was that miRNA-122 was downregulated. Given previous documentation that miRNA-122 binds to the hepatitis C genome to support replication, researchers postulated that the miRNA could also be used as an Ebola virus regulator.

The 20 genes identified (*CLDN3*, *ILF2*, *ILF3*, *NDUFA12*, *RUVBL2*, *SLC38A5*, *ACCN1*, *CEBPE*, *CRHR2*, *FAM63A*, *HMP19*, *IL2RA*, *LTF*, *PSMA1*, *RCHY1*, *SLC9A7*, *AC009283*, *LOC100289371*, *LOC440871*, *miR-122*) distinguished between the survivors and nonsurvivors. The researchers also identified a larger predictive gene set (238 genes) that distinguished between the two groups. This larger gene set was associated with inflammatory response in the host, T cell death, and inhibition of viral replication (Garamszegi *et al.*, 2014).

Three transcriptional molecules in the gene set were significantly enriched: CCAAT/enhancer-binding protein- α

(CEBPA), tumor protein 53 (*p53*), and megakaryoblastic leukemia 1 (*MKL1*) and myocardin-like protein 2 (*MKL2*) (Garamszegi *et al.*, 2014).

The findings in the study by Garamszegi *et al.* presents valuable evidence that further investigation of the transcriptional regulators noted above could reveal gene signatures that are specific to the Ebola virus infection and could allow for new treatments. The National Institutes of Health (NIH) has announced that it will begin the first clinical trial of an experimental adenovirus vector vaccine in which two Ebola genes have been inserted, prompting production of protein (Healy, 2014). The NIH hopes that the vaccine will produce an immune response in the human test participants. It will be imperative to ascertain whether the clinical trial reveals any new genetic biomarkers of disease outcome.

Despite promising research on new treatments for the Ebola virus infection, the rate at which vaccines are developed depends largely on public funding. Developing vaccines for infectious diseases is incredibly costly, with billions of dollars needed for research, clinical testing, and distribution. Given the relatively low number of infected humans in North America and Europe, many drug companies may not see the development of a viable vaccine for Ebola virus to be lucrative. The United States has funded most of the research, but this has been largely due to fears of the disease being used for biological warfare and terrorism.

Because the countries most affected by the outbreak are less developed, it becomes the responsibility of the more developed countries to cooperate to help curb the spread of infection.

At this time there has been some amount of cooperation among developed countries with regard to vaccine research—the NIH, for example, is working with GlaxoSmithKline, a British drug manufacturing company, to develop an experimental vaccine (Healy, 2014)—but a larger global response for humanitarian and containment efforts is needed.

There is a desperate need for the United Nations (UN) to be a driving force in coordinating emergency response to the epidemic. The United States, Great Britain, and Canada have all pledged funds and emergency workers to the relief effort, but there are not enough workers from each country alone to keep up with the rapid pace with which the infection spreads.

The New York Times reports that UN Secretary-General Ban Ki-moon had called a meeting with member nations to discuss emergency response plans on September 25, 2014 (Landler, 2014). It remains to be seen whether these talks will lead to an increase in global relief efforts.

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