VIRAL IMMUNOLOGY
Volume 28, Number 1, 2015

Pp. 1–2
DOI: 10.1089/vim.2015.1501

Editorial

Focus on Ebola Virus Research

William E. Dowling[1] and Robert J. Hogan[2]

In 2014, Ebola virus disease (EVD) emerged as a major international public health issue that garnered worldwide attention. The largest recorded Ebola virus outbreak in history began in Guinea in December 2013 and has spread throughout the neighboring nations of Sierra Leone and Liberia, with additional travel-related cases in Nigeria, Mali, Senegal, Spain, and the United States. At the time of writing, the World Health Organization has reported that there have been 17,290 EVD cases (10,825 lab confirmed) from this outbreak resulting in 6,128 deaths. In addition, an unrelated EVD outbreak was reported in August in the Democratic Republic of the Congo, resulting in 66 cases (38 lab confirmed) and 49 deaths. There are currently no approved vaccines or treatments for EVD, although there are several investigational products that are being accelerated into clinical trials through the efforts of multiple nations and pharmaceutical companies. This special issue of *Viral Immunology* is devoted entirely to Ebola virus research, and includes articles on human cases of EVD, nonhuman primate disease models, molecular mechanisms of virus immune evasion, cellular responses to infection, and novel vaccines.

The appearance of EVD in Guinea was the first reported instance of a filovirus infection in West Africa aside from a single nonfatal case reported in Ivory Coast in 1994 (Tai Forest Virus). However, the article by Boisen *et al.* from the Viral Hemorrhagic Fever Consortium (VHFC) suggests that humans in Sierra Leone have been exposed to Ebola viruses prior to the current outbreak. The VHFC has been treating Lassa Fever patients at the Kenema Government Hospital (KGH) in Sierra Leone since 2002. The majority of blood samples collected from patients clinically diagnosed with Lassa tested negative for Lassa virus, even though the patients all met the case definition of Lassa Fever and most were very ill. In a set of samples collected between 2011 and 2014, there were no significant differences in clinical signs and symptoms or in clinical chemistry measurements between patients who tested positive or negative for Lassa. Some were positive by enzyme-linked immunosorbent assay or reverse transcription polymerase chain reaction (RT-PCR) for other pathogens, including dengue virus, chikungunya virus, West Nile virus, *Leptospira*, *Rickettsia*, and *Plasmodium falciparum*, which demonstrates the wide range of infectious agents in the region. However, the most remarkable finding is that 22% of the patient samples tested positive for Ebola virus-specific immunoglobulin G, and all of these positive samples predate the current outbreak. In addition, one patient admitted in late 2011 was PCR positive for Ebola virus, albeit with a weak signal. These data suggest that in the context of other endemic diseases with similar presentation, EVD may have been in the region for some time but gone unrecognized.

A second paper in the issue focuses on EVD in humans. A short report by McElroy *et al*. is the third in a series of publications by this group analyzing samples from survivors and nonsurvivors of the 2000–2001 outbreak of Sudan virus in Gulu, Sudan, for biomarkers that predict clinical outcome. In their previous work, they identified that sCD40L correlated with survival and also reported that pediatric patients had higher levels of the adhesion molecules sICAM and sVCAM, with significantly higher levels in fatal cases compared to survivors. In this report, they found that von Willebrand factor (vWF), a protein important for platelet adhesion to wound sites, is elevated in Sudan virus–infected individuals, associated with fatal outcomes in pediatric patients, and correlates with hemorrhagic manifestations of disease irrespective of age. The authors speculate that the high levels of vWF are released by activated endothelial cells, consistent with increased levels of other markers of endothelial activation, and that this excessive endothelial activation contributes to poor outcomes in pediatric Sudan virus cases.

The current special issue also features two review articles. The first by Audet *et al*. focuses on the mechanisms employed by Ebola and other members of the family *Filoviridae* to evade the immune system. Ebola viruses are able to shut down pathways leading to interferon (IFN) production in infected cells as well as turning off IFN responses, mostly through the action of two viral proteins, VP24 and VP35. The VP35 protein can bind to dsRNA to prevent signaling through host pathways that are normally activated by dsRNA, leading to production of IFN-α and -β, including the protein kinase R (PKR) and the RIG-1 pathway. VP35 also acts upon the transcription factor IRF7 to prevent the expression of IFN, both by preventing IRF7 phosphorylation and facilitating its SUMOylation. The VP24 protein can interfere with the JAK-STAT cellular signaling pathway by interacting with karyopherin-α1, thereby preventing STAT1 from entering the nucleus to facilitate transcription of IFN-stimulated (IS) genes. The VP24 protein has also been shown to inhibit IFN signaling by disruption of the cellular MAPK pathway. Other viral proteins involved in immune evasion are the full-length glycoprotein ($GP_{1,2}$) and the soluble glycoprotein (sGP), which are produced from different transcripts of the GP gene via viral RNA-dependent

[1]Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, Rockville, Maryland.
[2]Departments of Veterinary Biosciences and Diagnostic Imaging and Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia.

RNA polymerase editing. For example, $GP_{1,2}$ has anti-tetherin activity, as well as the ability to block Fas-mediated apoptosis. In addition, sGP has a potential function in immune evasion, and this mechanism is thoroughly discussed in the second review article by De la Vega *et al*. sGP is found in large quantities in the bloodstream of infected animals, and several functions have been hypothesized. One major role of sGP is thought to be that of an immunological ''decoy,'' which can adsorb antibodies raised against $GP_{1,2}$, and this is supported by data showing that antibodies generated during infection cross-react with both forms of the viral GP. There are also data suggesting that sGP may potentially act as a structural protein, and it may play an anti-inflammatory role, as well as roles in lymphocyte apoptosis and neutrophil inactivation. However, these proposed functions remain controversial and will require further investigation.

It has been demonstrated in previous studies that Ebola viruses infect dendritic cells (DCs) and disrupt their function. In a third report investigating human–Ebola virus interaction, Melanson *et al.* outline and discuss the transcriptional profiles of human DCs infected with Ebola virus *in vitro*. Compared to uninfected controls, they noted increases in expression of several classes of genes, including cytokines, chemokines, antiviral, anti-apoptotic, and MHC I and II related genes. Of particular importance was the lack of an increase of expression of T-cell co-stimulatory and lymph-node homing receptor genes, which is consistent with the deficiencies that occur in DC function during Ebola virus infection in some animal model systems.

The other three reports in this issue involve studies in animal models of EVD. Rodent models require adaptation of the virus to cause disease, with the exception of immunocompromised mice. Nonhuman primates can be infected by viruses isolated from human clinical specimens resulting in a fatal disease with features similar to that observed in humans. A report by Martins *et al.* presents the clinical course of EVD in rhesus macaques ($n = 18$), including extensive pathological and immunological data. The animals were exposed intramuscularly to 1,000 plaque forming units of Ebola virus, and 17 of the 18 died or were euthanized based on clinical score between days 6 and 12 post-exposure. The animals displayed many of the hallmarks of EVD seen in humans, including fever, high viral load, coagulation abnormalities, decreases in platelets and reticulocytes, increases in multiple clinical chemistry measurements (AST, ALT, ALKP, BUN, CREAT, CRP), increase in inflammatory cytokines (Eotaxin, IP-10, MCP-1, and IL-6), and lymphopenia. The 18th animal survived infection and displayed a similar disease course to those that succumbed. However, there was a delay in the appearance of detectable viral RNA in this animal, as well as a delay in the inflammatory cytokine and chemokine responses. The authors speculate that these events may have contributed to the ability of the animal to control the infection and that therapies that act early to suppress viral load may therefore be a viable approach to help survival. Indeed, the cumulative results suggest an inverse relationship between the levels of serum cytokines and time to disease and subsequent death.

Using animal models, such as the ones employed by several of the groups that contributed to the current special issue, have resulted in several promising Ebola virus vaccines. Two such candidates are currently in Phase I clinical trials. Both involve recombinant viral vectors that express Ebola GP. The first one, developed by GlaxoSmithKline in collaboration with the U.S. National Institute of Allergy and Infectious Diseases, is a recombinant defective adenovirus-vectored vaccine expressing GP that uses an Ad3 viral vector derived from chimpanzees. The second is based upon a replication-competent, recombinant vesicular stomatitis virus (VSV) with GP replacing the VSV surface GP (VSVΔG/EBOV GP), which was developed by the Public Health Agency of Canada (PHAC). The studies by Alimonti *et al.* at PHAC seek to investigate this promising vaccine candidate further by elucidating the role that natural killer (NK) cells play in protection after immunization with VSVΔG/EBOV GP. Utilizing the adapted Ebola virus mouse model, the authors demonstrate that depletion of NK cells leads to a significant decrease in the time to death compared to nondepleted mice in the absence of vaccine. When the vaccine was given 24 h post-exposure, the presence of NK cells correlated with partial protection and increased mean time to death in nonsurvivors compared to NK-depleted mice. In addition, NK cell mediated cytotoxicity and IFN-γ secretion were significantly higher with VSVΔG/EBOV GP treatment. These data suggest that NK cells play an important role in protection conferred by VSVΔG/EBOV GP.

Another vaccine platform that has shown promise is based upon virus-like particles (VLPs). VLPs are produced by co-transfection of cells with DNA plasmids expressing GP and another viral structural protein, VP40. The article by Martins *et al*. describes a novel VLP vaccine that contains regions from Ebola virus, Sudan virus, and Marburg virus to create a trimeric, ''pan-filovirus'' vaccine. The authors show that expressed recombinant GPs containing the GP2 of Marburg virus and the N- or C-terminal regions of Sudan or Ebola GP1 can form VLPs when co-transfected with VP40, as demonstrated by Western blotting and electron micrographs showing particles that resemble filovirus morphology. Both chimeric VLPs conferred protection against Marburg virus challenge in the guinea pig model, but protection against Ebola virus depended on the design of the vaccines, with one VLP protecting 75% of the animals and the other 0%. The authors demonstrated that higher antibody titers resulted from incorporation of the C terminal region of GP1 compared to the N terminal region, and this correlated with the protection observed. These data demonstrate that GP2 and the C-terminal region of GP1 are highly immunogenic. More importantly, these findings should be taken into account for future vaccine design. They also suggest that a pan-filovirus vaccine based on chimeric GP is possible. Considering that there have been multiple outbreaks in the last 5 years involving disparate filoviruses from multiple species, this would be a substantial accomplishment.

In closing, the current special issue of *Viral Immunology* represents a wide spectrum of research on Ebola virus in the context of human infection and immune responses, animal models of disease, and further development and understanding of promising vaccine candidates. We hope the data presented herein will aid the research community and others, increase scientific and clinical knowledge, and help facilitate a more rapid end to the human suffering caused by the current Ebola virus outbreak.