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Review Article

[1]Department of Microbiology & Immunology, McGill University, Montreal, QC, Canada

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Email Correspondence

janna.shapiro@mail.mcgill.ca

Janna Shapiro[1]

Understanding the 2013-2015 Ebola Outbreak

Abstract

Background: The 2013-2015 Ebola outbreak caused severe human suffering and a global health crisis. Ebola Virus (EBOV) is a naturally zoonotic RNA virus that has several immune-evasion mechanisms and can cause serious disease and death in humans. The massive impact of the recent epidemic is unique in the 40-year history of this pathogen. Scientists and public health officials around the world are researching the factors that may have contributed to the scale and devastating nature of the 2013-2015 outbreak.

Methods: Terms searched online through the McGill library and Medline Ovid included "Ebola", "immune evasion", "sequencing", "Ebola glycoprotein" and "zoonotic transmission". Only articles published since 2014 were selected.

Summary: In this review article, we will provide discussion on the principal factors contributing to the unusually destructive nature of the 2013-2015 Ebola outbreak. Interestingly, although several nonsynonymous mutations have been observed in the recently circulating strains, they were not the principal cause of the unusually devastating nature of the outbreak. Instead, the high rate of transmission was likely caused by sociological factors, such as population dynamics and late detection of the outbreak. However, there is evidence to suggest that once the high rate of transmission in humans was established there was selective pressure on the virus to evade the human immune system. This selective pressure may have exacerbated an already deadly outbreak. Ongoing research efforts indicate that there is still much to be discovered about the virus and the control of outbreak management.

Introduction

The 2013-2015 outbreak of Ebola Virus Disease (EVD) had 28,637 reported cases and 11,314 deaths in West Africa, with widespread transmission in Guinea, Liberia and Sierra Leone. (1) Several other regions have also been affected, with cases in other parts of Africa, Europe, and the United States. (1) In comparison, in the 24 previously recorded outbreaks from 1976 to 2013, a total of 2,400 cases were reported, with only seven of these outbreaks resulting in greater than 100 reported cases. (2) The unprecedented scale of the 2013-2015 Ebola outbreak has raised many questions in scientific and public health communities about the factors which contributed to the virulence and high level of human-to-human transmission that characterized this outbreak. The long-term impacts of EVD on survivors as well as the affected regions are still unclear.

Early sequencing data from strains circulating in Guinea suggested that a single introduction event from an unknown reservoir was the source of the outbreak. (3) This strain of EBOV had not been previously defined, and was named "Makona", after the Makona River situated at the border of Liberia, Guinea, and Sierra Leone. (3) In addition to Makona, a second strain, named "Lomela" was associated with approximately 70 cases of EVD in the Democratic Republic of the Congo. (3) Research suggests that fruit bats may be the natural reservoir of EBOV; however many other organisms, such as gorillas and chimpanzees have been found to harbor the virus. (4, 5) The identification of the unknown natural reservoir of EBOV and the characteristics that differentiate Makona from previous EBOV strains will continue to be important in gaining a more complete understanding of EVD.

EBOV, also known as *Zaire Ebolavirus*, is a negative-sense single-stranded RNA virus of the Ebolavirus genus of Filoviridae family. (6) Of the 5 species in the Ebolavirus genus, *Zaire Ebolavirus* is the member species, and there are four other species in the *Ebolavirus* genus including the most virulent. (7) The RNA genome codes for seven genes that are processed into eight proteins. (8) The ribonucleoprotein complex is made up of the viral genome and viral proteins nucleoprotein (NP), large structural protein (L) (the RNA-dependent RNA polymerase), and viral proteins 35 (VP35) and 30 (VP30). (8) The viral envelope is derived from the host cell surface, which has been lined with viral proteins 40 and 24 (VP40/VP24), the matrix proteins. (8) The glycoprotein gene codes for a transmembrane protein, called GP or $GP_{1,2}$, and a soluble glycoprotein called sGP. (9) GP is cleaved after translation to form the GP_1 and GP_2 subunits, and allows the virus to interact with host cells. (9) Specifically, GP_1 mediates attachment to the host cell, while GP_2 mediates fusion. (9) The exact role of the, (sGP), is unknown. (6)

Two mechanisms have been described which allow EBOV to evade the human immune system during infection.(6) The first mechanism is mediated by VP35 and VP24. (6) These viral proteins inhibit type 1 interferon (IFN) production and signaling, an important part of the innate immune response against viruses. (10) VP35 binds to double-stranded (ds) RNA as well as the 5' cap structure to protect the viral dsRNA from being recognized by cellular sensors of foreign RNA. (10) As depicted in Fig. 1, this prevents the activation of the retinoic acid inducible gene I (RIG-I) pathway and therefore the production of IFN-α and IFN-β, which are necessary in establishing an early immune response. (11) VP24 interferes with karyopherin-1α, the protein that transports signal transducer and activator of transcription 1 (STAT1) into the nucleus. (12) There is also evidence that VP24 interacts directly with STAT1. (13) When STAT1 is inhibited from entering the nucleus, it cannot activate transcription of the genes needed for an effective IFN response (Fig. 2). (11) Both VP24 and VP35 are necessary to attenuate IFN signaling and effectively evade the immune response. This IFN antagonism is of great importance as it allows EBOV to be persist in its human host.

The second major immune evasion mechanism used by EBOV is mediated by the viral glycoproteins. (14) sGP has been found to reduce the amount of antibody production specific to $GP_{1,2}$ by stimulating the production of antibodies that cross-react with sGP and $GP_{1,2}$. (9) Additionally, $GP_{1,2}$ has a "steric shielding effect" that blocks the major histocompatibility complex 1 (MHC-1), β1-integrin, and FAS from detection by the immune system. (15, 16) These surface proteins are important for infected cells to interact with the cells of the immune system, so their inaccessibility in the case of steric shielding greatly inhibits the host's ability to protect against viral infection. (15, 16) GP_2 subunit can also inhibit the cell's ability to prevent

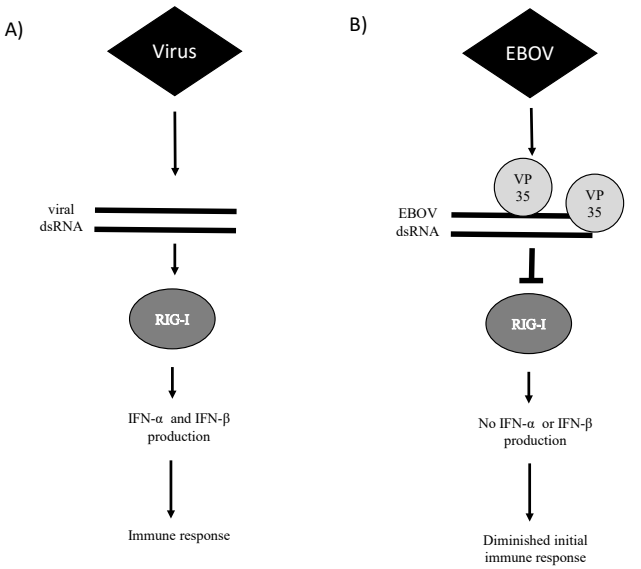

Fig. 1: VP35 Immune Evasion. (A) Normal RIG-I pathway. RIG-I senses double stranded RNA in the cell and promotes the expression of interferon-α and interferon-β (5). (B.In a cell infected by EBOV, VP35 binds to viral double stranded RNA at the phosphate back-bone and the 5′ cap to prevent RIG-I from sensing the infection (4).

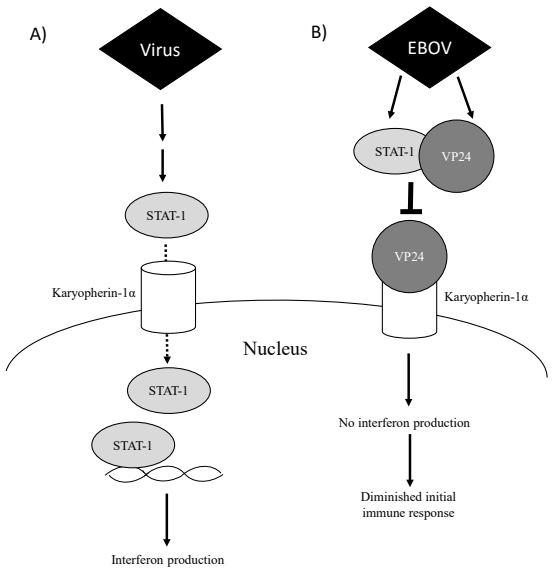

Fig. 2: VP24 Immune Evasion. (A) Normal STAT-1 pathway. Viral infection activates STAT-1, which enters the nucleus and acts as a transcription factor to activate interferon production (5). (B) In a cell infected by EBOV, VP24 binds to STAT-1, preventing it from entering the nucleus. VP24 also interacts with karyopherin-1α, further preventing STAT-1 translocation into the nucleus (4).

viral release by moving the tetherin in the cell. (17) Tetherin is a transmembrane glycoprotein that retains viral particles in the infected cell and is therefore an anti-viral mechanism. (17) Through its interaction with tetherin, GP_2 allows EBOV to evade this host defense strategy. (17) Hence, it is likely that both sGP and $GP_{1,2}$ contribute to the pathogenicity of EBOV in humans.

In addition to immune evasion, EBOV infection results in severe consequences for the human host. Although the clinical course of EVD is well known, the mechanisms associated with EBOV pathogenicity are not well defined. EBOV is able to invade almost all host cells, but early replication is thought to occur primarily in macrophages and dendritic cells. (18) The virus destroys infected cells, thus inhibiting several crucial physiological systems, such as the immune system. (19) This is yet another immune evasion strategy of EBOV. In particular, infections become fatal when the inflammatory response is dysregulated, leading to systemic inflammation. (20) In addition, EBOV has been shown to decrease endothelial-cell barrier function and affect the synthesis of blood coagulation protein in the liver. (21) These host-virus interactions are central to the clinical course of EVD.

In the aftermath of the 2013-2015 Ebola outbreak, it is evident that the mechanisms underlying the severe pathogenicity and rapid spread of the virus are not completely elucidated. These questions are especially perplexing when comparing the scale of this most recent outbreak to the other EBOV outbreaks over the past 40 years. This review article suggests that evolution of EBOV leading up to and during the 2013-2015 epidemic contributed only minimally to the severity of the outbreak.

Results

Evolution Rates Between and Within Outbreaks

A logical place to start in forming an understanding of the virulence of the 2013-2015 Ebola epidemic is a comparison between the circulating strains during the most recent epidemic and strains found in previous, less severe epidemics. In a study by Gire and colleagues, they sequenced 99 Ebola virus genomes from 78 patients in Sierra Leone in 2014. (22) When compared to all previously published EBOV sequences, these strains contained 341 fixed substitutions, including 35 nonsynonymous, 173 synonymous and 133 noncoding mutations. (22) In 2015, Park *et al.* sequenced and analyzed 232 EBOV genomes from Sierra Leone and compared them to the 78 genomes that had been previously reported in 2014. (23) In the 232 sequences analyzed, 85 contained at least one intra-host single-nucleotide variant (iSNV). Interestingly, several iSNVs were found in two or more sequences. (23) iSNVs are important because they can be tracked from patient to patient and provide information about chains of transmission. (22) The presence of iSNVs shared between samples indicates that the transmission bottleneck allowed for a mutation acquired in one host to be transferred to another. (23) This sequencing data was a crucial first step in determining whether mutations in specific regions of the EBOV genome contributed to its high virulence in the 2013-2015 outbreak.

Comparing the genomes originally sequenced by Gire *et al.* to those later sequenced by Park *et al.* provided significant insight as to the impact of large-scale human-to-human transmission of EBOV. The substitution rate observed by Gire *et al.* was found to be almost twice as high, with more nonsynonymous mutations within the 2014 outbreak, compared to the rate of evolution since the emergence of EBOV. This early evidence suggested that the progression of the epidemic allowed the virus to adapt. (22) Park *et al.* challenge these findings and, specifically, the high reported rate of nonsynonymous mutations.(23) The authors found that in contrast to the original estimations by Gire *et al.*, the evolution rate of Makona was similar to the long-term evolution rate observed between outbreaks. (23) This phenomenon was attributed to purifying selection whereby mutations that have a deleterious effect on protein structure or function are eliminated. (24) Purifying selection is observed when there are more synonymous mutations than nonsynonymous in the genome. (24) In the case of EBOV from 2013-2015, although a higher than expected rate of nonsynonymous mutations was found early in the outbreak, these mutations were often deleterious, and impaired viral fitness. (23) As a result, they were transmitted to new hosts with a low frequency and were often only observed in a single individual. To visualize this phenomenon, Park at al. created a phylogenetic tree of derived alleles at genomic position 18.911. (23) The deleterious mutations occur on the external branches of this phylogenetic tree. (23) On internal branches of the phylogenetic tree, there are mutations present in multiple samples. (23) In these internal branches, nonsynonymous mutations accumulated at a much lower rate compared with synonymous mutations. As the epidemic progressed most of the mutations that were sustained in multiple hosts were synonymous, since if the mutation was sustained, then the virus was fit enough to replicate and therefore did not suffer any major deleterious nonsynonymous mutation. (23) This may explain why

the initially high rate of evolution during the outbreak did not continue further into the epidemic. Although the evolutionary rates found in these two studies contradict each other, both studies suggest that there were several nonsynonymous mutations in the Makona strain, when compared to EBOV strains isolated in previous outbreaks. (22, 23) It was hypothesized that these mutations may have contributed to the high virulence of the Makona strain.

Evolution of the IFN Antagonism System Between Outbreaks

A study by Dunham and colleagues in 2015 built on the genetic sequence data provided by the Gire and Park studies. (25) Their research assessed whether the nucleotide substitutions that differentiated the VP24 and VP35 genes of the Makona virus from the prototype strain, Mayinga, increased viral fitness or the ability to inhibit the host IFN response. (25) The authors used a monocistronic minigenome system as a model of viral replication and transcription. (25, 26) This allowed them to measure whether the Makona VP24 and VP35 proteins had an increased ability to function as a cofactor for the viral RNA polymerase, thus resulting in an overall increase in viral fitness. (25) By measuring transcription and replication rates, they found that the VP35 and VP24 proteins from Makona virus had indistinguishable effects on genome replication from the prototype virus. (25)

In a second experiment Dunham *et al.* tested VP35 and VP24's IFN interferon antagonism. (25) The ability of VP35 to inhibit IFN-β production was measured with cells that were transfected with a reporter plasmid under the control of an IFN-β promoter and various concentrations of a VP35 construct. (25) Reporter gene expression was stimulated by co-transfection of plasmids containing the caspase activation and recruitment domain (CARD) of RIG-I. (25) The CARD domain is constitutively active and stimulates the the production of IFN through the IFN-β promoter. (25) A similar experiment was carried out to assess VP24's IFN antagonism. In this experiment a reporter plasmid under the control of an IFN stimulated response element, which was stimulated with human IFN-β, was used. (25) For both VP24 and VP35, there was no statistical difference in function between the Makona and Mayinga variants in IFN antagonism. (25) Thus, these results suggest that none of the nonsynonymous mutations investigated provide Makona virus with an increased fitness over the Mayinga virus, with respect to either EBOV replication or IFN antagonism.

Evolution of Glycoprotein

Two studies investigated the effect of evolution of the EBOV GP on Makona virulence. (23, 27) In a study by Azarian *et al.*, 65 glycoprotein sequences from epidemic waves between 1976 and 2014 were used to reconstruct the evolutionary history of EBOV. (27) This analysis found that, over time, the evolution of EBOV has been driven by neutral genetic drift, demonstrated by the similar rates of synonymous and non-synonymous mutations. Furthermore, specific amino acid substitutions were found to be mostly transient, rather than established in the population. (27) This indicates that over a large time scale, there has been little selection acting on the EBOV glycoprotein. This may be because GP is implicated in host cell attachment and fusion. (27) Therefore, mutations in this protein may affect the virus' ability to infect host cells making it impossible for the virus to survive.

A second analysis, carried out by Park *et al.* focused on a shorter timescale to determine the effect of human-to-human transmission on the EBOV genome. (23) They found that although the general rate of nonsynonymous mutations decreased as the outbreak progressed, this was not true for the mucin-like domain of the EBOV GP. (22) The authors compared the rate of nonsynonymous to synonymous mutations, both during the outbreak and between outbreaks, for all of the major proteins in EBOV. They found that the mucin-like domain of the glycoprotein was the only region in which the log of the rate was greater than 1. This implies that both between and within outbreaks, there were more nonsynonymous substitutions than would be expected under neutral genetic drift. (23) According to the authors, the higher rate of nonsynonymous substitutions suggests that the evolution of GP, the primary target of host antibodies, may be subject to faster than usual evolution and diversifying

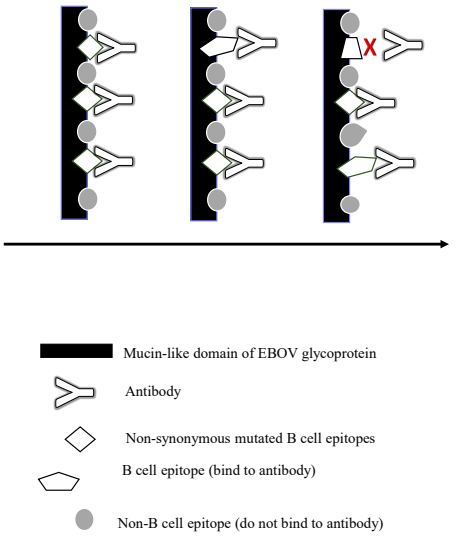

Fig. 3: Evolution of the EBOV Glycoprotein During the Epidemic. A schematic representation of the evolution of different regions of the mucin-like domain of the EBOV glycoprotein from September 2014 to June 2015. Non-synonymous mutations occurred more often than under neutrality only in the B-cell epitopes. This indicates that, under the selective pressure of the human immune system, EBOV may have evolved to evade host antibodies (8).

selection. In the context of virus-host interactions, this could change the antibody-binding sites on GP and allow the protein to evade the host humoral immune response. To test this hypothesis, the authors needed to determine whether the nonsynonymous mutations occurred within regions of the mucin-like domain that are bound by antibodies. Park *et al.* used the Virus Pathogen Database to find experimentally determined sequences of B cell epitopes. (23, 28) They found that there were in fact more nonsynonymous mutations in the regions of GP that bind to antibodies would be expected by chance (Fig. 3). (23) This finding supports the idea that the selective pressure acting on the virus is the human humoral immune response and that due to extensive human-to-human transmission, the EBOV evolved to better evade the host immune system.

Sociological Factors

Other studies in Ebola viral dynamics have argued that further genomic sequencing will not allow us to understand the severity of the recent outbreak. (27) Instead of changes in viral phenotype, research points to human behavior, population dynamics, and late detection of the outbreak as important contributing factors.

It can be argued that changes in land use and cultural practices have increased human exposure to zoonotic hosts, making an outbreak more likely. (29) Specifically, forested land which previously blocked human-animal contact, is now being used for agriculture, industry, and residential areas, which do not afford the same protection from zoonotic reservoirs of EBOV. (29) Further increasing this risk of exposure are practices such as bush meat hunting and burial traditions. (30) As zoonotic transmission events become increasingly possible, EBOV has the ability to infect more and more people. Furthermore, since it is believed that Ebola virus is harbored in many animal hosts, there is a possibility of various strains from different animals being introduced into human populations. (4, 5)

Other research points to rapidly changing population dynamics in areas where zoonotic transmission is possible as a factor contributing to the scale of the recent outbreak. (30) Over the past 40 years, the proportion of people living in urban areas has increased from 25.5% to 59.2% in the predicted zoonotic niche of EBOV. (30) As people live in closer proximity to each other, the likelihood of viral transmission increases. (30) Furthermore, populations are significantly more interconnected than they

were in 1976. During the eight-year period from 2005-2012 alone, global airline passenger volumes increased by one third. (30) Importantly, increased mobility has made it easier for the virus to be transmitted across national borders.

A final factor that has been explored is the impact of late detection of the zoonotic transmission event and the subsequent human-to-human transmission. The early symptoms of EVD are non-specific, making it difficult for health practitioners to recognize early cases as being EVD. (31) Furthermore, poor health infrastructure and epidemiological surveillance systems in place in Western Africa contributed to late detection. (31) In analyzing the response times in previous Ebola outbreaks, it is not surprising that when the outbreak is not detected early, there is a greater likelihood that it will migrate from rural to urban areas. (31) While it is difficult to quantify the effects of these various sociological factors, changes in behavior, land use, and undeveloped public health systems likely contributed to the scale of the 2013-2015 EBOV outbreak.

Discussion

Genetic Variation

The major conclusion that can be drawn from these results is that no major mutations of the EBOV have been identified that allowed it to spread more quickly and cause greater human suffering compared to the previous 24 recorded Ebola outbreaks. However, sustained transmission afforded the virus an unprecedented opportunity to adapt in the human host, leading to some mutations in GP involved in immune evasion. (23) This indicates that evading the human immune system may be the pressure that is driving evolution. Since this pressure requires sustained transmission to have a lasting effect on the viral genome, it cannot be the initial cause of the high rate of transmission or what dictated the unprecedented scale of this outbreak. Once the outbreak was underway however, it is possible that the mutations in this domain may have exacerbated the spread of disease and contributed to the Makona strain's pathogenicity and transmission. Furthermore, if the virus has in fact evolved to be more pathogenic during this outbreak, future outbreaks may be increasingly destructive.

The idea that the initial scale of the outbreak was not due to a mutation is supported by four main studies that investigated EBOV sequences in the 2013-2105 outbreak (Makona) and previous outbreaks (Mayinga). (22, 23, 25, 27) While the Gire and Park studies focused on comparing overall rates of synonymous to nonsynonymous mutations, the Azarian and Dunham studies focused on the proteins involved in EBOV immune evasion. Taken together, their findings demonstrate that the evolution of the EBOV has been generally driven by neutral genetic drift. Furthermore, between outbreaks, there is no known selective evolutionary pressure on the virus to become more pathogenic to humans, and thus

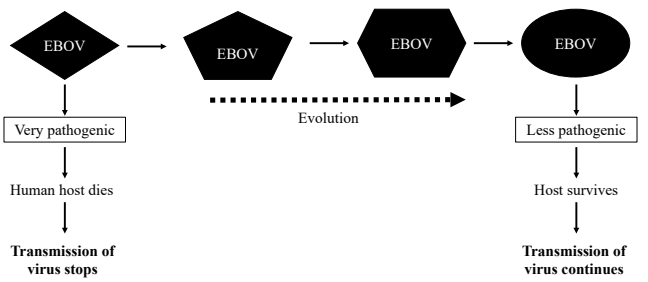

Fig. 4: The Effect of Extended Human-to-Human Transmission of EBOV- Dead-End Host Concept. Humans are a dead-end host for EBOV. If the virus is too pathogenic, it will kill the host and the chain of transmission will be broken. Therefore, over the course of an epidemic, natural selection will select a virus that is less pathogenic, so the host can survive and continue transmission, thus ensuring survival of the virus (9).

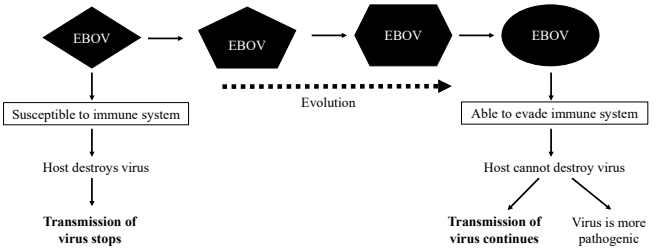

Fig. 5: The Effect of Extended Human-to-Human Transmission of EBOV – Immune Evasion Concept. If EBOV is susceptible to the host immune system, the virus will die and transmission will stop. Therefore, over the course of an epidemic, natural selection will select for a virus that is better able to evade the immune system, so transmission can continue (8). However, this selective pressure also makes the virus more pathogenic to the host.

there have been no sustained mutations that have lead to a hyper-virulent strain of EBOV.

The continued human-to human transmission of EBOV observed during the recent outbreak was unprecedented for this virus. (23) There is currently contention within the scientific community as to the effects of this sustained transmission. Dunham *et al.* argue that conceptually, since sustained human-to-human transmission is not characteristic of EBOV, evolutionary pressure would select a less pathogenic virus, thus increasing the chances that the host survives and can transmit the virus (Fig. 4). (25) This was supported by their findings that neither VP24 nor VP35 of the Makona strain have an increased ability to aid in viral transcription or replication, or to interfere with the host IFN response. (25) On the other hand, Park *et al.* argue that there is a selective pressure on the virus to evade the host immune system. (23) While the main reason for this evolutionary pressure is to ensure the survival of the virus, as a by-product, these mutations may also make the virus more pathogenic (Fig. 5). This argument was supported by the higher than expected rate of non-synonymous mutations observed in the mucin-like domain of GP. This indicates the possibility of host antibodies driving the selection of altered B-cell epitopes, which inhibit the humoral immune response. (22) However, as the authors noted, these findings were based on a very small sample size. Furthermore, since the B-cell epitopes used in the analysis were not determined from an in vivo study, they may not be immunodominant. (23) Although the selective pressures that arose during the outbreak are still not completely understood, elucidating these mechanisms may yield critical information in understanding past outbreaks and managing EBOV in the future.

Continued genetic analysis of EBOV is necessary to understand the factors that made the recent Ebola outbreak so destructive. Due to the high level of pathogenicity of the virus and the geographical location of the outbreak, research has been slow and laborious. Park *et al.* suggest that an important research topic in the immediate future is to develop methods to deactivate the virus while maintaining the integrity of the sample so that it can be used for high-quality genomic sequencing. (23) The ultimate goal of continued research efforts is to be able to prevent the transmission and the suffering associated with EVD.

Sociological Factors

The evidence that none of the mutations in the viral genome over the past four decades can explain the scale of the 2013-2015 epidemic supports the idea that sociological factors played a critical role. (27) To assess the impact of these factors, we must determine if there has been a change since previous EBOV outbreaks and if this change affected viral transmission patterns. For example, although there were changes in land use that lead to increased exposure to zoonotic hosts, this was unlikely to affect viral transmission. This is because epidemiological analysis of the 2013-2015 outbreak suggests that there was a single event that introduced the virus into humans. (22) Therefore, though changes in land

use may have allowed for the initial transmission event, this does not account for the scale of the outbreak.

On the other hand, changes in population dynamics did lead to the possibility of "superspreading events". For example, the burial of a single patient in Sierra Leone is linked to 300 EVD cases. (2) Furthermore, in contrast to the recent urban Ebola outbreak, past outbreaks began in isolated rural areas and did not spread to urban settings. (31) This allowed for more effective outbreak-control strategies. (31) Based on the above evidence, it is likely that urbanization and mobility significantly contributed to the high number of cases of EBOV during the 2013-2015 outbreak. Finally, late detection has a significant impact on the effectiveness of control interventions.(31) Once the virus has travelled to a densely populated area, it becomes increasingly difficult to control. (30) This suggests that along with changing population dynamics, late detection of the outbreak had a large impact on the level of Ebola transmission.

Conclusion

In conclusion, analysis of the 2013-2015 Ebola outbreak indicates that sociological conditions, rather than genetic mutations, were the main factors contributing to the unprecedented scale and impact of the outbreak. While mutations during sustained human-to-human transmission may have exacerbated the outbreak, the initial causes were likely the increase in urban populations and late detection of the zoonotic transmission event. Therefore, research into how Ebola virus infects hosts, how it evades the immune system and the sociological factors surrounding EVD are vital to controlling future outbreaks.

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