**Mini Review**

# Ebola Virus Disease: Updated Pathophysiology and Clinical Aspects

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**Abstract**

The aim of this article is to review and update the pathophysiological mechanisms and clinical aspects of the Ebola virus disease including the novel therapeutic measures. Ebola virus, a RNA virus, was discovered in 1976 as " *Zaire ebolavirus* " and currently is responsible for outbreak in the west Africa. The mucin-liked region of the Ebola virus envelope play a significant role in viral infection in non-human primates and humans through attachment of the membrane anchored C-type lectins rather than specific receptors. Both humoral and cellular immunities are responsible for the survival. Several novel chemotherapeutics are developed and progressive, such as ZMapp, PMOs, BCX-4430, AVI-602, T-705, TMK-Ebola, CMX-001, etc.Health education and counseling of the communities should be implemented, particularly, distance education to reduce the burden of the Ebola-virus-disease stigmatization. Nevertheless, strategic prevention of this virus is the most significant control measures.

## ABBREVIATIONS

DNA :Deoxyribonucleic Acid, GP: Glycoprotein, IFN: Interferon, IL: Interleukin, IP: Inducible Protein, kDA: kiloDalton, KO: Knocked Out, M-CSF: Macrophage-Colony Stimulating Factor, MIF: Migration Inhibitory Factor, MIP: Macrophage Inhibitory Protein, NK: Natural Killer, PPE: Personal Protective Equipment, NPC1: Cholesterol Transporter Protein Niemann-Pick C1, RNA: Ribonucleic Acid, RT-PCR: Reverse Transcriptase-Polymerase Chain Reaction, sGP: Soluble Glycoprotein, Tim-1: T-cell immunoglobulin and mucin domain, TNF: Tumor Necrosis Factor, US CDC: United States Centers for Disease Control and Prevention, VP: Viral Protein

## INTRODUCTION

The virus is classified into the family "*Filoviridae*" and genus "*Ebolavirus*" [1]. There are five species of Ebola virus : *Reston ebolavirus*, *Taï Forest ebolavirus*, *Sudan ebolavirus*, *Bundibugyo ebolavirus*, and *Zaire ebolavirus* [2,3].*Zaire ebolavirus* was discovered in 1976 and is responsible for the current outbreak in the west Africa [2,4]. The filamentous and pleomorphic Ebolavirus is enveloped non-segmented negative strand RNA virus of 19 kb in length with a mean unit length of 1,200 nm [3,4]. Each of its five species is pathogenic for humans except *Reston*

*ebolavirus* that only has been demonstrated to be pathogenic for nonhuman-primates [3]. This enveloped virus consists of a lipid bilayer coat that protects the virus genome and facilitates its host-cell entry [3]. The viral genome encodes for RNA dependent RNA polymerase, four structural proteins namely VP24, VP30, VP35, and VP40, a glycoprotein (a soluble 60- to 70-kDa protein and a full-length 150- to 170-kDa protein [4]), and a nucleoprotein [3,4]. The viral heavy glycosylation and the lipid content of the viral envelope allow the immune evasion [3]. The natural reservoir of virus remains unknown [3,4], nevertheless, the little collared bat ( *Myonycteris torquata* ) is believed to be the most likely reservoir [3].

## PATHOPHYSIOLOGIC MECHANISMS

Tissue invasion by the Ebola virus occurs via the infected fluid that contacts with the mucosal or skin breaks [3], and preferably replicate in the monocytes, macrophages, and dendritic cells [2]. *In vitro* studies, the viral envelope glycoprotein is responsible for both receptor binding and fusion of the viral envelope with the host cell membrane [3]. The viral envelope is heavily glycosylated that includes both N- and O-linked glycan, which protects the host-immune attack [3]. Recent studies indicated that cysteine protease, likes cathepsins B and L, promote the viral glycoprotein-host cell membrane fusion [3]. An association

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between apoptosis and fatal outcome has been identified both in vitroin infected human cells and in vivo in mouse and nonhuman primate models [5]. A previous study by McElroy et alin 4 Ebola-virus-disease survivors demonstrated that there were activated CD4- and CD8-T cells in all survivors, with approximately 30-60% of CD8-T cell expressing activation markers [5]. The three severe cases demonstrated peak plasmablast frequencies between 2 and 3 weeks after onset of symptoms, whereas another case with very-mild-disease and rapid resolusion developed the peak plasmablast levels 3 days after the onset of symptoms, but not the same magnitude as the more severely ill cases [5]. Surprisingly, activated CD8-T cell elevation remained in all three severe cases, in contrast to the case with very-mild-disease whose activated CD8- and CD4-T cell levels increased early in the course of illness, but declined to the baseline as the illness rapidly resolved [5]. Additionally, the three severe cases had a second peak of activated CD8- and CD4-T cell levels, possibly representing the return of the tissue-based T cells to the peripheral blood after achieving the viral control in the affected tissues [5]. Declining of the viral load among three severe cases began during their second week of illness, coincidently with the presence of the activated T- and B-cell elevation [5]. Two severe cases still had high levels of activated CD4- and CD8-T cells at the first follow-up appointment (approximately 2 months post symptom onset),that suggested ongoing antigen stimulation via the T-cell receptor, whereas one severe case and the case with very-mild-disease demonstrated the baseline levels of the activated T cell at their first follow-up visit (71 days after symptom onset, later in the disease course than the follow-up visits for the other two severe cases) [5]. In contrast, the patient with very-mild-disease and rapid resolution of the viremia, had low numbers of activated CD4- and CD8-T cells both at the hospital discharge and the first follow-up visit [5]. Due to a wide range of cell- lineages targeting, it is difficult to define the specific mechanisms (receptors) of viral entry into such cell types [3]. When the virus triggers expression of a host of pro-inflammatory cytokines, including interleukin (IL)-2, IL-6, IL-8, tumor necrosis factor α, interferons (IFNs), and interferon inducible protein, the severe disease-progression occurs [2]. The mucin-like region which is rich in glycosylated residues of the Ebola virus envelope play an important role in viral infection of the monocytoid lineages, hepatocytes, and endothelial cells and is believed to involve membrane anchored C-type lectins that can be attachment factors rather than specific receptors [3]. The soluble glycoprotein(sGP) of the Ebola virus may contribute to the immune evasion by inhibiting the early steps of the neutrophil activation that would assist in virus clearance [4].

## IMMUNOGENIC MECHANISMS

McElroy et al also demonstrated in their study that all 4 survivors revealed detectably specific IgG response as early as 2 days after the symptom onset and developed peak IgG responses around 2-3 weeks after symptom onset, that consistent with their plasmablast responses [5]. Nevertheless, one severe case and the case with very-mild-disease developed both IgM and IgG responses at the same week, whereas the other two severe cases developed the classic kinetics of an IgM response the IgG response [5]. The majority of their specimens revealed predominantly IgG-positive plasmablast [5]. Interestingly, two severe cases still had 5-15% Ebola virus-specific plasmablast at the time of their first follow-up visit [5]. When whole-cell lysate was used rather than the viral nucleoprotein alone, there was higher magnitude

of the antigen-specific IgG responses, indicating multiple viral antigen targets [5]. A significant proportion of the IgG-producing plasmablasts that was not antigen-specific was still demonstrated, indicating activation of the polyclonal B cells that is mediated by the inflammatory response [5]. The CD4 T-cell responses were lower in magnitude compared to the CD8 T-cell responses, but were much more diverse in terms of antigenic specificity [5]. IFN system, one of the major innate immune responses that is counteracted by the Ebola virus, has been demonstrated to inhibit the synthesis of host cell IFN-inducible transmembrane proteins 1-3, tetherin and other virus restricting molecules that could serve as barriers against the virus [3]. Monocyte and macrophage infection with virus contributes to increased synthesis of tumor necrosis factor (TNF)-α [3,5], release of IL-1, IL-2 [5],IL-6, IL-8, IL-15, IL-16, eotaxin, IP-10, M-CFS, MIF, MIP-1 α and β, and contributes to lymphoid cell apoptosis, the characteristic of Ebola virus infection [3]. The depletion of NK cells abolishes the Ebola virus protection [3]. A murine model study revealed that mice with KO strains deficient in CD8+ T cells did not survive infection, while those deficient in CD4+ T cells survived indicating the role of cytotoxic T cells in protection against Ebola virus [3]. The same results were demonstrated in nonhuman primate studies [3].

## PATIENT'S HISTORY

Two main factors of initial evaluation of a patient with suspected Ebola infection are arrival from, living or working in endemic area in the past 21 days and history or presence of a fever in the past 24 hours [2]. Persons who work with high risk clinical samples or with bats or primates are also at high risk [2]. In symptomatic patients, use of personal protective equipment (PPE) and precautionary isolation procedures are mandated [2].

## CLINICAL MANIFESTATIONS

In the 2014 outbreak, the most common symptoms reported between symptom onset and case detection were fever (87.1%), fatigue (76.4%), vomiting (67.6%), diarrhea (65.6%), loss of appetite (64.5%), headache (53.4%), abdominal pain (44.3%), and unexplained bleeding (18%) [2]. Maculopapular rash can develop in early stage, approximately 25-52% [6]. Lymphadenopathy has been rarely reported [2]. In advanced stage of Ebola virus disease, multiple organ dysfunctions is common that includes liver damage, pancreatitis, acute renal injury, and adrenal failure [2]. Serum level of aspartate aminotransferase is higher than the serum level of alanine aminotransferase indicating hepatitis [2]. In late stage of renal involvement, the Ebola virus can directly damage to the kidneys or may be disseminated intravascular coagulation [2]. In advanced Ebola virus-infected cases, they usually reveal hypotension, tachycardia, hiccup, hepatosplenomegaly, confusion, and seizures [2]. In fatal cases, massive gastrointestinal bleeding is frequently found [2].

## INVESTIGATIONS

Reverse transcriptase polymerase chain reaction (RT-PCR) is the main confirmatory test [2]. Ebola viral RNA can be detected in the blood by the RT-PCR from day 3 to days 6-17 of the symptoms [2]. If the RT-PCR test reveals negative, the test should be repeated within 48 hours [2]. Other useful investigations include Ebola virus specific IgM and IgG antibodies, serum amylase, coagulation studies, renal function tests, liver function tests, blood cultures, chest radiography, arterial blood gases, antigen capture-enzyme-linked-immunosorbent assay tests, and complete blood count [2].

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## INFECTION CONTROL

Isolation of patients identified as being at risk of infection must be immediately performed in a room with private bathroom facilities, while all attending healthcare personnel must wear PPE [2]. All specimens for laboratory investigations must be collected and sent off [2]. To reduce the risk of transmission and needlestick injuries, judicious selection of investigations and early placement of a central lineare needed [2].

## GENERAL AND SYMPTOMATIC MANAGEMENT

In patients with mild dehydration, oral rehydration can be used [2]. Fluid replacement more than 10 litre/day should be administered in febrile cases with diarrhea [2]. In cases with signs of shock and fluid losses, the volume of intravenous fluid needed should be assessed on the basis of clinical examination [2]. Daily monitoring of the serum electrolyte levels should be performed [2]. In cases with hypoperfusion, high serum lactate levels are the reliable measure for fluid resuscitation need [2]. Renal replacement therapy has been administered in anuria cases without response to fluid resuscitation need [2]. Platelet and plasma transfusion should be administered in advanced cases with major bleeding [2,7]. Broad spectrum antibiotics ( such as meropenem, piperacillin-tazobactam, or ceftriaxone ) should be included in cases with septic shock or sepsis in the first hour after sending the blood cultures, appropriate airway management and oxygen administration, urine output monitoring, and rapid intravenous fluid resuscitation [2]. Inotropic support with a central venous catheter in an intensive care unit where invasive monitoring enables more aggressive corrections of fluids, acid-base balance, and electrolytes, should be considered in cases without response to the initial management [2].

## EMERGING TREATMENTS

AVI-7537 consists of antisense phosphorodiamidate morpholinooligomers (PMOs) that target the Ebola virus VP24 gene , whereas AVI-602 consists of two PMOs (AV-7537 and AV-7539), which targets the VP35 gene [2]. BCX-4430 is an adenosine analogue that is active against Ebola virus in rodents by inhibition of viral RNA dependent RNA polymerase of paramyxoviruses, arenaviruses, bunyaviruses, and flaviviruses [2]. FAvipiravir or T-705 selectively viral RNA dependent RNA polymerase of the foot and mouth disease virus, alphaviruses, bunyaviruses, arenaviruses, flaviviruses, yellow fever virus, West Nile virus, and influenza viruses [2]. TKM-Ebola consists of a combination of small interfering RNAs that target Ebola virus RNA polymerase L, formulated with lipid nanoparticle technology [2]. Brincidofovir or CMX-001 demonstrated activity against Ebola virus *in vitro* [2]. These mentioned compounds will be undergone clinical trials for Ebola virus treatment soon [2]. Amiodarone, interferons, chloroquine, and clomiphene inhibit Ebola virus interactions with human cells in models will be in clinical trial soon [2].

## VACCINES AGAINST EBOLA VIRUS

The ideal candidate vaccine is able to confer interspecies cross-protection against Zaire ebolavirus, Bundibugyo ebolavirus, Sudan ebolavirus, and unknown Ebola virus species [8]. A recent study demonstrated the possible potential for developing the cross-protective vaccines for the Ebola viruses [9]. Important preventive vaccines include human parainfluenza virus 3 that revealed 100% protection following a single vaccination in guinea pigs, but it required 2 vaccinations to induce protective immunity in non-human primates [10] and rabies virus-recombinant Ebola virus vaccine that demonstrated 100% of protection in mice model following challenge with Zaire Ebola virus [11]. Naked plasmid DNA, a gene-based approach, has been used successfully in animal models to control the synthesis of immunogens within the host cells [5]. The plasmid DNA immunization that was developed in the guinea pig was the first successful vaccine for Ebola virus infection [5]. Only sGP and GP elicited T-cell proliferative and cytotoxic responses, and humoral response [5]. The cytotoxic effects of GP on endothelial cell function and macrophage disrupt the inflammatory function and the integrity of the vasculature [5].The protection was conferred by each of these immunogens when the animals were infected within one month of the last immunization [5]. It is unclear whether the attenuated murine virus is more susceptible to neutralization than the wild-type virus, thus, the relative potency of the nucleoprotein, as an immunogen for providing long-term protection, remains uncertain [5]. DNA vaccines efficacy in humans or nonhuman primates is less compared to the efficacy in rodents [5]. The dysregulation of the inflammatory response and the vascular dysfunction characteristic of the lethal Ebola virus infection provide a rationale for focusing on GP as a target for a protective vaccine [5]. The most effective strategies for therapeutic vaccines is the use of selectively monoclonal antibodies with high neutralizing potential [3]. Currently, two experimental vaccines are undergoing trials [2]. One trial is cAd3-ZEBOV, a chimpanzee derived adenovirus vector with an Ebola virus gene inserted [2]. Another trial is rVSV-ZEBOV, an attenuated vesicular stomatitis virus with one of its genes replaced by an Ebola virus gene [2]. These pre-clinical trials are performed in the United States, United Kingdom, Switzerland, and some African countries, whereas the clinical trials have been launched in the United States [2]. ZMapp, the best known therapeutic vaccine, is a combination of three humanized monoclonal antibodies targeted at three Ebola virus glycoprotein epitopes [2]. ZMapp had been proved protective to rescue 100% when administered to non-human primates, particularly rhesus macaques, 24-72 hours after infection for initiation up to 5 days post-challenge [2,3,12]. Despite its potential, numbers are too small to conclude about its safety and efficacy [2]. More doses are needed to conduct larger clinical trials [2].

## CONVALESCENT PLASMA OR WHOLE BLOOD

The evidence from past Ebola outbreaks that transfusion of blood from convalescent cases, thought to contain naturally specific-protective antibodies developed during the disease might be beneficial in the acute phase of infection and may decrease the mortality, with some success [8,13]. Unfortunately, the convalescent plasma has not been repeated in further outbreaks due to in vitro studies demonstrating the antibodies without neutralizing activity against the Ebola virus [8]. Additionally, they failed to protect the nonhuman primates although monoclonal antibodies to the GP of the Ebola virus demonstrated protective and therapeutic effects in mice [8].

## OTHER MEASURES OF CONTROL

The rapid case identification and promptly forceful intervention can stop the virus transmission [14]. In current

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Ebola virus disease outbreaks, under-resourced African regions not only suffer from a critically low ratio of health-care workers to total population, but also lack essential PPE to practice standard infection control measures, and also lack the infrastructure and local capacity essential to effectively trace the contacts and isolate infectious persons [15]. Socio-cultural factors in these regions, particularly, touching the body of the decease greatly allow the dissemination of the Ebola virus [15].

## DISCUSSION

Because of the wide range of cell-lineages targeting of the Ebola virus, the identification of specific mechanisms of viral entry into human cells is difficult [3]. It is clear that the viral envelope glycoprotein (GP), which includes both O- and N-linked glycans is responsible for both fusion of the viral envelope with the host cell membrane and receptor binding [3]. Cholesterol transporter protein Niemann-Pick C1 (NPC1), the T-cell immunoglobulin and mucin domain (Tim-1), members of the tyrosine receptor kinases (Ax1, Dtk and Mer), and the GPI-anchored cell surface expressed folate receptor-αare the tools as the receptors implicated utilizing pseudotyped-Ebolavirus-GP containing viruses [3]. Both O- and N-linked glycans of the viral envelope serve as a shield against host immune attack and, thus, the virus can escape the immune effector mechanisms [3]. These glycans can generate the non-neutralizing antibodies against disposable and highly variable regions of the Ebola virus envelope, whereas the sites of major glycosylation are localized to the middle third of the glycoprotein envelope [3]. These sites of major glycosylation are referred as the mucin-like region [3]. The mucin-like region of the Ebola virus envelope play an important role in viral infection by human-cell attachment via membrane anchored C-type lectin involvement [3] and finally inhibit the neutrophil activation [4]. There were data that supported the development of a strong antigen-specific T-cell response against Ebola virus during infection, but the correlation of this response to the patient outcome remains to be investigated [5]. Due to identification of the major T-cell target during human infection, future T cell-based vaccine designs could benefit from including the viral nucleoprotein antigen [5]. Several previous studies revealed that both humoral and cellular immunities involved in survival. Several agents have been the emerging treatments for the Ebola virus disease, whereas ZMapp is currently the best known one, but larger clinical trials are needed [2]. There was some evidences that the convalescent plasma or whole blood could be beneficial in the acute Ebola-virus infection, nevertheless, further studies should be conducted [13].

## CONCLUSION

During the acute period of illness, Ebola virus is shed in a wide variety of bodily fluids, but the risk of transmission from fomites in an isolation ward and from convalescent patients is low when presently recommended infection control guidelines for the Ebola virus are followed. Strategic measures of disease prevention are still the key success for Ebola-virus-disease control, while individual treatments with the novel agents, particularly ZMapp are necessary for whom developed severely clinical manifestations. Health education and counseling of the communities associated with the Ebola-virus-disease survivors should be implemented, particularly, distance education to reduce the burden of the Ebola-virus-disease stigmatization.

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