Ebola Virus and its Mechanism in Fatal Disease Formation

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Abstract:

  Recently Ebola Virus, its infection, disease development, fatality rate and safety issues has turned into buzzwords in the world especially to the people of the West African countries where the rigorousness of the disease varies from asymptomatic response (mild illness) to rapid fatality. The fashion Ebola infection keeps an eye on the demolition of immune system having severe consequences. Ebola GP binding receptor, Cholesterol transporter NPC1 (Niemann–Pick C1) protein paves the way of infection occurrence along with TIM-1 (T-cell Ig and Mucin Domain 1) expression. After infection, immune cells transport the virus to the nearby lymph nodes for further replication

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and thus crafting mature virus progeny for fatal disease formation. In a nutshell, perceiving the mechanism of infection and replication of this viruses could be better options of molecular and nano-medicine development against EVD (Ebola Virus Disease) in the next decades.

Key words: EHF (Ebola Hemorrhagic Fever), GP (Glycoprotein), EVD (Ebola Virus Disease)]

INTRODUCTION

Ebola virus, once designed as Zaire Ebola virus but now is also known as EBOV, is in point of fact a fatal disease causing agent which can be deliberated as a killer virus due to having its wide-ranging virulence activity and deficient medication opportunities. Ebola virus disease (EVD) with severe hemorrhagic fever occurring not only in humans but also in many other mammals and the mortality rate is high in Ebolavirus genus (Kuhn et al. 2010). Actually Ebola virus and its genus was firstly found in Democratic Republic of Congo and named after nearby African river Ebola where first hemorrhagic fever had been found. Organ damage, multi-organ dysfunction, diffused intravascular coagulopathy (DIC) with platelets and coagulation factor consumption which are responsible for hemorrhage (Dr. Jonathan Gubbay et al., 2014). The majority of human deaths from EVD in recent years reaching an epidemic nature specifically in the West African countries including Sierra Leone, Guinea, Liberia, and Cote d'Ivoire with the infection of more than twenty seven thousand Ebola viral victims and about twelve thousand deaths. Most outbreaks were small, but the virus captured the attention of the world due to death rates as high as 90% as well as the visceral manner in which it killed (Johnson, K.M. & Breman, J.G., 1978). Ebola virus is so-called to have very close relation with Marburg virus (Pattyn et al., 1977). Like Baculo virus, Ebola is passed on by vector. In case of Ebola virus, not only

precise fruit bats but also chimpanzee can accomplish as natural reservoir (Quammen and David, 2014). Tropical rain forests of Africa continent are best-suited for Ebola virus. Infection of EBOV varies from person to person. Generally it is transmitted between humans (usually people who come in close contract) and sometimes, from animals to humans through blood fluids contaminated with the virus of interest. Ebola virus replicates preferentially in monocytes/macrophages and dendritic cells which facilitate dissemination of the virus throughout the body via lymphatic system (Mupapa et al., 1999). So, dangerous and hazardous sample handling make Ebola virus research a burning question but still today different sophisticated laboratory based diagnosis are undertaken (Murphy , 2002).

Symptoms start 2 days to 3 weeks after contracting the virus with fever, sore throat, muscle pains, headaches, nausea, vomiting and diarrhea followed by decreased hepato-renal functioning (Angier and Natalie, 2014). Abnormal low concentration of lymphocyte is a common subsequence of Ebola virus invasion. In some extent it may destroy our immunity completely at the severe stage which is almost similar with HIV infection. Recently a multivalent vaccine candidate (EBO7) has been developed that expressed the glycoproteins of Zaire ebolavirus (ZEBOV) and Sudan ebolavirus (SEBOV) in a single complex adenovirus-based vector (CAdVax) and showed potential protection of non-human primates against those ebolaviruses (Pratt, 2010).

Classification of Ebola virus

The genus Ebola virus of family Filoviridae contains five species namely Zaire virus (EBOV), Sudan virus (SEBOV), Ivory coast virus or Tai forest ebolavirus (TEBOV), Bundibugyo virus (BEBOV) and Reston virus (REBOV). The mortality rate for Ebola viral infection is almost 83% to 90% (WHO, 2014). The outbreaks and epidemics as well as the mortality range of

each of these species are given below in table 1. Among the viruses, Reston virus causes infection in animals but others causes human infection.

Table 1.Outbreaks and epidemics and mortality range of human due to various species of Ebola virus (Mike Bray & Daniel S Chertow, 2016).

Species name	Outbreaks and epidemics	Mortality range (Human)
Zaire virus	Multiple outbreaks in central Africa, 2014-2015 west Africanepidemics.	55-88%
Sudan virus	Four epidemics up to date- two in Sudan in 1970, one in Uganda in 2000 and again in Sudan in 2004.	Approximately 50%
Tai forest ebolavirus	In Ivory coast in 1994.	Less severe than Zaire and Sudan virus.
Bundibugyo virus	In Uganda, 2007.	Approximately 30%
Reston virus	Outbreaks in Reston due to lethal infection of Macaques imported into USA in 1989 from Philippines, three more outbreaks occurred in USA and Europe before outbreaks in Philippines	Low pathogenicity or non-pathogenic

The genome of Ebola virus

Recently the 'Ebola Issue' has become a major concern for the matter of medication of EVD and also for the complex genetic constitution of Ebola virus. Almost 99 Ebola virus genomes were sequenced where about 300 differences from virus genomes were responsible for past outbreak. Actually EBOV carries a linear, single-stranded, negative-sense RNA genome (possesses 18,959 to 118,961 nucleotides in length & 4.2×10^6 Da in weight) with other components such as viral envelope, matrix and nucleocapsid components in virions. Being polymorphic, the virion reveals 'U'- shaped, '6'-shaped, Shepherd's crook-shaped, 9-shaped, Eye bolt-shapes, Coiled shapes or other forms after purification and visualization through electron microscope. The whole cylindrical shape

retains approximately 80 nm (sometimes can be 800 nm to 1000 nm) in diameter (Muyembe-Tamfum et al., 2011 & Klenk and Feldmann, 2004). Nucleocapsid is composed of a series of viral proteins attached to an 18–19 kb linear, negative-sense RNA without 3′-polyadenylation or 5′-capping and the RNA is helically wound and complexed with the NP, VP35, VP30, and L proteins. This helix has a diameter of 80 nm and contains a central channel of 20–30 nm in diameter. Ebola virus genome transcribed into 8 sub-genomic mRNA proteins where, seven of them are considered as structural proteins and one is non-structural protein namely sGP or small glycoprotein whose function is unknown (Dr. Jonathan Gubbay et al., 2014). Structural proteins are (Feldmann, 1993) -

Nucleoprotein (NP) - it is associated with transcription and replication.

Glycoprotein (GP) – it projects 7-10 nm long spikes from its lipid bilayer surface. Individual GP molecules appear with space of about 10 nm. It is associated with membrane development. Secretory Glycoprotein binds to antibody and contains possible anti-neutrophil activity.

RNA dependent RNA polymerase (L protein) - it is associated with transcription and replication.

Virion proteins (VP35, VP40, VP30 & VP24) - these proteins are associated with membrane. VP35 & VP40 act as polymerase cofactor whereas VP30 & VP24 act as transcription activator. Viral proteins VP40 and VP24 are located in the matrix space between the envelope and the nucleocapsid.

The Pathway of Ebola Virus replication

Ebola use a combination of host and virally encoded enzymes alongside host cell structures to produce multiple copies of them (Biomarker Database, 2009). 472 nucleotides in the 3′ end and 731 nucleotides in the 5′ end are sufficient for the replication of a viral mini genome (Klenk and Feldmann, 2004). Ebolavirus

attach with the host cell surface receptors through glycoprotein peplomer. Afterwards it undergoes a cellular mechanism named endocytosis in the host cells (Saeed et al., 2010). The vesicle membrane is fused with the viral membrane causing cellular penetration and release of viral nucleocapsid into the cytoplasm of host cell. Encapsulated, negative-sense genomic ssRNA is used as a template for the synthesis (3'-5') of polyadenylated, monocistronic mRNAs and through using the host cell's ribosomes, tRNA molecules, etc., the mRNA is translated into individual viral proteins. Using negative-sense genomic RNA as a template, a complementary (+)ssRNA is synthesized and used as a template for the synthesis of rapidly encapsulated new genomic (-)ssRNA. Cellular enzymes and substrates are highly utilized to commit glycosylation of a vital glycoprotein precursor GP0 (it is cleaved to GP1 and GP2).
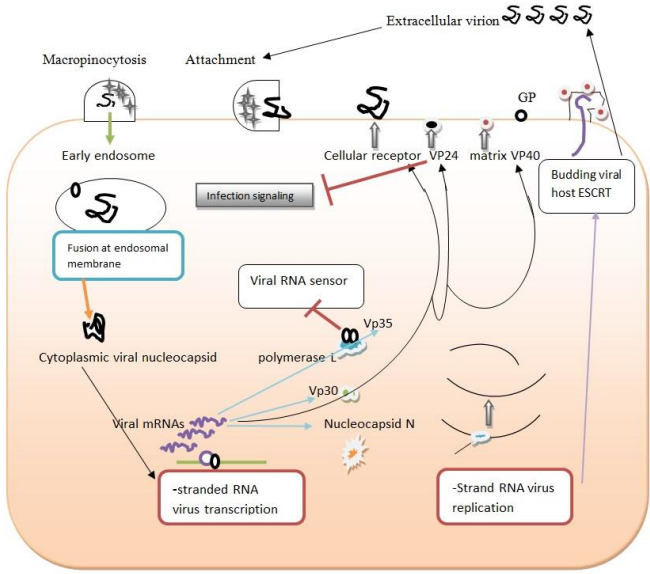

Fig.1 Ebola virus genome replication mechanism.

The infected cell releases both the secreted glycoprotein and delta peptide synthesized generally from cleaved glycoprotein precursor. Finally, nucleocapsid proteins and envelop proteins

undergo budding and new virions are released through destroying the cell membrane (Fig. 1).

Risky sources of infection

Ebola appears in human body through close contact with the blood containing Ebola virus, body fluids, and secretions of infected animals such as chimpanzee, gorillas, fruit bats, monkeys, porcupines, forest antelope etc. found ill or dead or in the rain forest causing Ebola virus disease (Lisa et al., 2002). Ebola virus disease has also resulted from accidental laboratory infections. So highly sophisticated safety cabinet is the precursor to remain safe during laboratory work. Airborne transmission between monkeys was demonstrated during the outbreak of Reston Ebola virus in Virginia, but there is limited evidence of airborne transmission in any human epidemic. Body fluids such as salica, mucus, vomit, feces, sweat, tears, breast milk, urine and semen may contain Ebola virus after infection (Bausch et al., 2007). In most people the virus spread through blood, feces and vomit (Donald, 2014). Entry points for the virus include the nose, mouth, eyes, open wounds, cuts and abrasions. Laboratory generated droplets of Ebola virus having 0.8–1.2 μm size are breathable because large droplets are spread (Johnson et al., 1995). Because of this potential route of infection, these viruses have been classified as Category A biological weapons (Leffel and Reed, 2004). Men who have recovered from the disease can still transmit the virus through their semen for up to 7 weeks after recovery from illness. Nosocomial infections have been frequent in Africa. Epidemic potential can spread from person to person, most often during the care of patients, which requires strengthening of strict infection control measures during the management of cases. All ages and genders are susceptible to the Ebola virus infection. The severity of the disease in humans varies widely, from rapid fatality to mild illness or even asymptomatic response (Gina Kolata, 2014). The Niemann–Pick C1 (NPC1) protein is a

cholesterol transporter protein and the main receptor for Ebola-GP binding and entry of Ebola virions into the host cell for replication (Carette JE et.al 2011). TIM-1 (T-cell Ig and Mucin Domain 1) was shown to bind to the receptor binding domain of the EBOV glycoprotein to increase the receptivity of Vero cells. Silencing its effect with siRNA prevented infection of Vero cells. A monoclonal antibody against the IgV domain of TIM-1 blocked EBOV binding and infection (Kondratowicz et al., 2011). The cells and tissues with high NPC1 and TIM-1 expression may be major sites of viral infection and sites of significant shedding of mature infectious viral progeny. This includes not only the antigen-presenting cells and macrophages in the airway and skin, but also from tissues with high TIM-1 expression levels that are known to be seriously affected by EBOV lysis. This conceivably could be a factor in human-to-human virus transmission events and transmission from infected patients to medical workers (Carette JE et.al 2011 & Kondratowicz et al., 2011).

Infection mechanism of Ebola Virus
Endothelial cells (cells lining the inside of blood vessels), liver cells, and several types of immune cells such as macrophages, monocytes, and dendritic cells are the main targets of infection. Following infection with the virus, the immune cells carry the virus to nearby lymph nodes where further reproduction of the virus takes place. From there, the virus can enter the blood stream and lymphatic system and spread throughout the body (Funk and Kumar. 2014). Macrophages are the first target to be infected by Ebola virus and thus programmed cell death appears (Chippaux, 2014). The continuous growth of Ebola virus glycoprotein synthesis and consistent breakdown of endothelial cells lead to injury to the blood vessels. The bigger concern for the Ebola patient is improper clotting and liver damage. The widespread bleeding that occurs in affected people causes swelling and shock due to loss of blood volume (Smith,

2005). The dysfunction in bleeding and clotting commonly seen in EVD has been attributed to increased activation of the extrinsic pathway of the coagulation cascade due to excessive tissue factor production by macrophages and monocytes (Goeijenbier et al., 2014). The sGP forms a dimeric protein that interferes with the signaling of neutrophils, another type of white blood cell, which enables the virus to evade the immune system by inhibiting early steps of neutrophil activation. The presence of viral particles and the cell damage resulting from viruses budding out of the cell causes the release of chemical signals (such as TNF-α, IL-6 and IL-8), which are molecular signals for fever and inflammation (Kühl and Pöhlmann, 2012 & Misasi and Sullivan NJ, 2014). Ebola virus can interfere with our innate immunity and cellular ability to generate interferon proteins (Olejnik et al., 2011 & Ramanan et al., 2011).
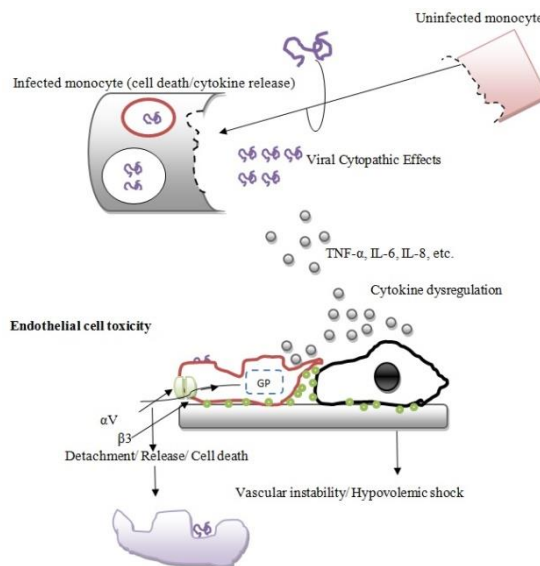

Fig. 2. Molecular mechanism of Ebola virus infection

The VP24 and VP35 structural proteins of EBOV play a key role in this interference. Disrupting our cell signaling system, suppressing immunity and synthesizing unnecessary molecules EBOV spreads throughout the body very quickly. A marked

elevation of interferon (IFN)-γ levels (>100 pg/mL) was observed in sequential serum samples from all fatal EHF cases compared with patients who recovered or controls. Markedly elevated serum levels of interleukin (IL)-2, IL-10, tumor necrosis factor (TNF)-α, and IFN-α were also noted in fatal EHF cases; however, they had a greater degree of variability. No differences were noted in serum levels of IL-4 and IL-6 (Francois et al., 1999). Ebola virus (EBOV) infections for EHF are characterized by dysregulation of normal host immune responses. Insight into the mechanism came from recent studies in nonhuman primates, which showed that EBOV infects cells of the mononuclear phagocyte system (MPS), resulting in apoptosis of bystander lymphocytes (Lisa et al., 2002).
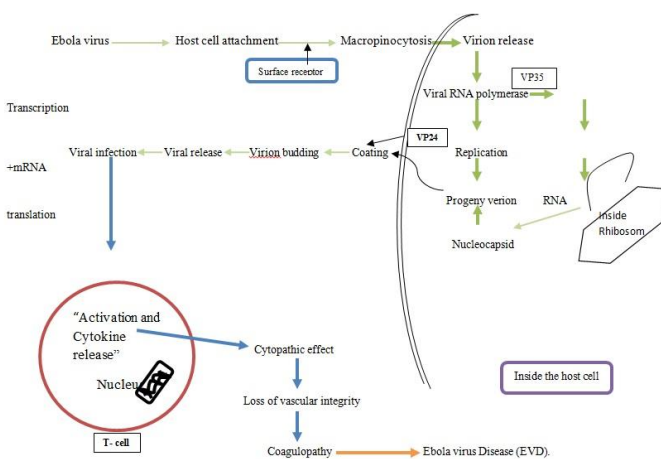

Fig. 3. The stages of Ebola virus infection and disease formation.

Small capillary vessels are the main target of Ebola virus infection. The virus attach to walls, cause leakage of blood and serum into surrounding tissue. When white blood cells attack the virus, they are dissolved and release a chemical into the blood stream that signals the release of other chemicals (pro-inflammatory cytokines, pro-coagulants, and anticoagulants). The injured blood vessels results in permanent bleeding.

Eventually, the entire body is leaked during Ebola virus infection (Murphy, 2002).

Consequences of infection

If the patients don't recover early, there is a high probability that the disease will progress to the second phase, resulting in complications which eventually lead to death (Mupapa et al., 1999). Patients who are progressed with phase two EHF almost always die (Ndambi et al., 1999). Multiple organ dysfunction syndrome (MODS) is the consequence of continuing severe systemic vascular inflammation with generalized increased capillary permeability, capillary leak, and edema (Johnson and Mayers, 2001). In MODS, organ dysfunction is precipitated by capillary changes in permeability, blood flow, and the development of micro-vascular stasis and micro-thrombi (Steven et al., 2014). Therapy therefore is limited to maintain adequate tissue perfusion and adequate tissue oxygenation. The chance of survival diminishes as the number of different organs involved increases, and the mortality rate of MODS has changed little since it's recognition in 1980s (Irwin et al., 2013). Along with the elevated body temperature, early complaints include nausea, vomiting, diarrhea, and loss of appetite (WHO, June 2014). Death due to MODS usually occurs within seven to 16 days (usually between days eight and nine) after the first symptoms, if the infected persons are not recovered. Skeletal muscle weakness is a very common symptom for Ebola virus infection (Wendo, 2001).

Directions for the future research on Ebola Virus and Proper medication

1. GP is a transmembrane glycoprotein. During the attachment process to the host cell, GP act as the ticket to enter into the cell. Ebola virus contains GP1 and GP2 proteins where "Anti-GP1" and "Anti-GP2"

proteins can be designed to restrict the infection procedure.

2. Anti VP35 development should be very handy to stop further damage after invasion.

3. Site specific mutagenesis in the cellular system to change the makeup of receptors so that macropinocytosis formation can be stopped after the viral attachment.

4. Proenzymes should be developed to insert into the host cell to lyse the virulence enzymetic system. Proenzyme actually stays in an inactive form after entering into the host cell but a certain time later it becomes activated as an enzyme. If it is designed with any monoclonal antibody then its activity would be point specific and more transparent.

5. Biosynthetically cytokine specific additional regulatory molecules can be engineered to avoid cytokine dysregulation after few days of infection.

6. Nano-sensing devices and nano-filtration systems can be designed using nanoparticles in the intra-cellular stage.

7. Specific viral infection signal tracing and killing the virulent, chimeric molecules can be developed using BAX (apoptotic gene) with bionano-particles.

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