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Review on Ebola Virus Disease in Clinical and Diagnostic Aspects

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Abstract

The 2014 outbreak clearly showed that Ebola viruses (EBOV) remain a substantial threat for public health. The mainstay of management of patients with Ebola disease is isolation of patients and use of strict barrier nursing procedures; the present treatment strategies are mainly symptomatic and supportive (fluid resuscitation, antiypyretics, antidiarrheal drugs). Currently, there is no approved therapy for Ebola hemorrhagic fever (EHF), however several advanced treatment options were tested in animal models (on non-human primates or rodents). They include use of both symptomatic (e.g. use of tissue factor inhibitors - rhNAPc2, rhAPC - to abolish coagulopathy) and specific antiviral approaches: e.g. monoclonal anti EBOV antibodies (ZMapp, MB-003), phosphorodiamidate morpholino oligomers (PMOs), liposomes containing siRNA (LNP-siRNA:TKM-Ebola) and small molecule inhibitors (e.g. BCX4430, favipiravir). The scope of this article is to briefly review the most promising therapeutics for EHF, based on the data coming from rare clinical reports, studies on animals and results from in vitro models. Several candidates of vaccines also were investigated their efficacy in animal models by National Institute of Health (NIH) and Department of Defense, and they are processing of clinical tests in West Africa aiming to finish the development by the 2015. Vaccine and therapeutic development is essential to stop the EVD outbreak in West Africa, also to protect the world from the risk which can be generated by potential spread of Ebola virus.

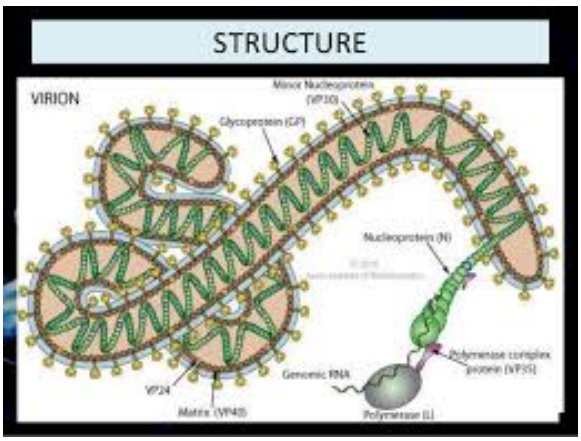
Keywords: Ebola virus, Vaccines, Drug development, Ebola hemorrhagic fever, treatment methods

INTRODUCTION

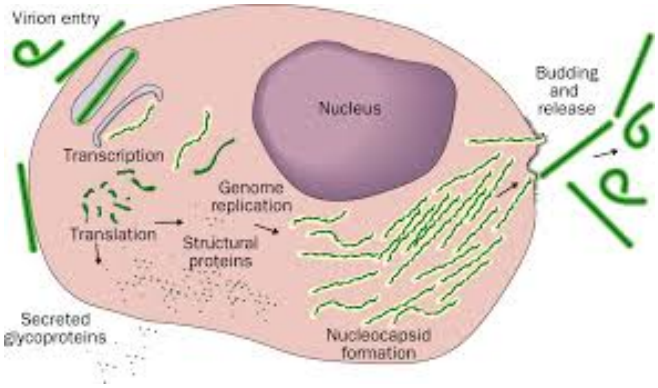
Ebola virus causes severe viral hemorrhagic fever with a high fatality rate. Five Ebola virus species within the genus Ebolavirus are known, including four that cause Ebola virus disease (EVD) in humans (a fifth species has only caused disease in nonhuman primates).1 The 2014 outbreak of EVD in West Africa, caused by Ebola virus (Zaire ebolavirus species), is the largest outbreak of EVD in history.2 Ebola virus can be transmitted by direct contact with blood, body fluids, or skin of EVD patients or persons who have died of EVD.3 As of October 23, 2014, 450 healthcare personnel are known to have become infected with Ebola, of whom 244 died.2,4 Several U.S. healthcare personnel working in West Africa have also become infected with EVD and have returned to the United States for evaluation and treatment.5 In addition, people in several states who have had recent travel to West Africa and have developed fever and other symptoms have been evaluated at U.S. hospitals for possible EVD. As of October 29, 2014, there have been two imported cases, including one death, and two locally acquired cases in healthcare workers reported in the United States.

‘‘Ebola,’’ however, is not just one Ebola: There are 4 distinguishable subtypes, whose phylogenetic tree is shown on page iii of this supplement [1–3]. Because the subtypes, which may even be different virus species, have differing properties, we have grouped the papers by the subtype discussed within each subject area.

On 8 August 2014 the World Health Organisation (WHO) declared the Ebola virus disease (EVD) outbreak in West Africa a Public Health Emergency of International Concern (PHEIC) [4] stressing the need for international attention and collaboration to control the outbreak. At this moment (18 September 2014) a total of 5335 cases with 2622 reported deaths have been notified, in Guinea, Liberia, and Sierra Leone. The imported EVD case in Nigeria that resulted in a relatively small outbreak, and similar imported cases in the USA and Spain which at first appeared to have been well contained, but eventually lead to infection of healthcare workers, show the importance of adequate isolation methods, training of personnel and the adequate use of personal protective equipment (PPE).[5] For the West Africa outbreak the total number of cases is subject to change due to ongoing reclassification, retrospective investigation and the availability of laboratory results. A second, non-related, EVD outbreak has been reported in the Democratic Republic of Congo with currently a total of 62 confirmed and suspected cases.[3,4]



Structure of Ebola virus



Molecular Biology

Transmission of Ebola virus disease (EVD)

Ebolaviruses enter the human body via mucosal surfaces, abrasions and injuries in the skin or by direct parental transmission. For each outbreak of EVD a single introduction from the animal kingdom is needed. It is likely that, as for the index case, infection occurs after human contact with primates, e.g. due to hunting or consuming of infected animals, while also other mammals such as antelopes and rodents have been mentioned as potential reservoirs.[6] Another potential cause for human infection was described in 2005 where data from a large study in bats showed three fruit bat species to be a potential reservoir for Ebolaviruses.[7] This was later confirmed by an EVD outbreak that resulted after direct contact with bats.[8, 9] Due to the high viral loads seen in the body fluids of EVD patients human to human transmission can easily occur. This transmission seems to take place through body fluid contact and not by airborne transmission (e.g. infective aerosols).

Stability and viability

In blood and or other body fluids, or on contaminated surfaces, EBOV can survive for hours at room temperature (20°C−25°C), and for weeks at low temperature (4°C) [17]. EBOV is only moderately heat resistance and can be inactivated by heat treatment (>60°C) for at least 1 hour. EBOV is also sensitive to ultraviolet light, gamma rays, and many chemical reagents, including ether, peracetic acid, sodium hypochlorite, and formaldehyde[43].

Emergency Department Processes for the Evaluation and Management of Persons Under Investigation for Ebola Virus Disease

Patients with epidemiologic risk for Ebola virus disease and symptoms consistent with Ebola virus disease are presenting to emergency departments (EDs) and clinics in the United States. These individuals, identified as a person under investigation for Ebola virus disease, are initially screened using a molecular assay for Ebola virus. If this initial test is negative and the person under investigation has been symptomatic for < 3 days, a repeat test is required after 3 days of symptoms to verify the negative result. In the time interval before the second test result is available, manifestations of the underlying disease process for the person under investigation, whether due to Ebola virus disease or some other etiology, may require further investigation to direct appropriate therapy.

ED processes for the safe and timely evaluation and management of the person under investigation for Ebola virus disease are presented with the ultimate goals of protecting providers and ensuring a consistent level of care while confirmatory testing is pending.[10-12]

Curcumin Suppression of Cytokine Release and Cytokine Storm. A Potential Therapy for Patients with Ebola and Other Severe Viral Infections

The activity of curcumin in suppressing multiple cytokines, and its activity in experimental models of diseases and conditions associated with cytokine storm, suggest it may be useful in the treatment of patients with Ebola and cytokine storm. Curcumin is poorly absorbed from the intestinal tract; however, intravenous formulations may allow therapeutic blood levels of curcumin to be achieved in patients diagnosed with cytokine storm. Clinical status and levels of important cytokines, such as IL1β, IL6 and TNFα, should be monitored carefully when patients are treated with curcumin.[13-17]

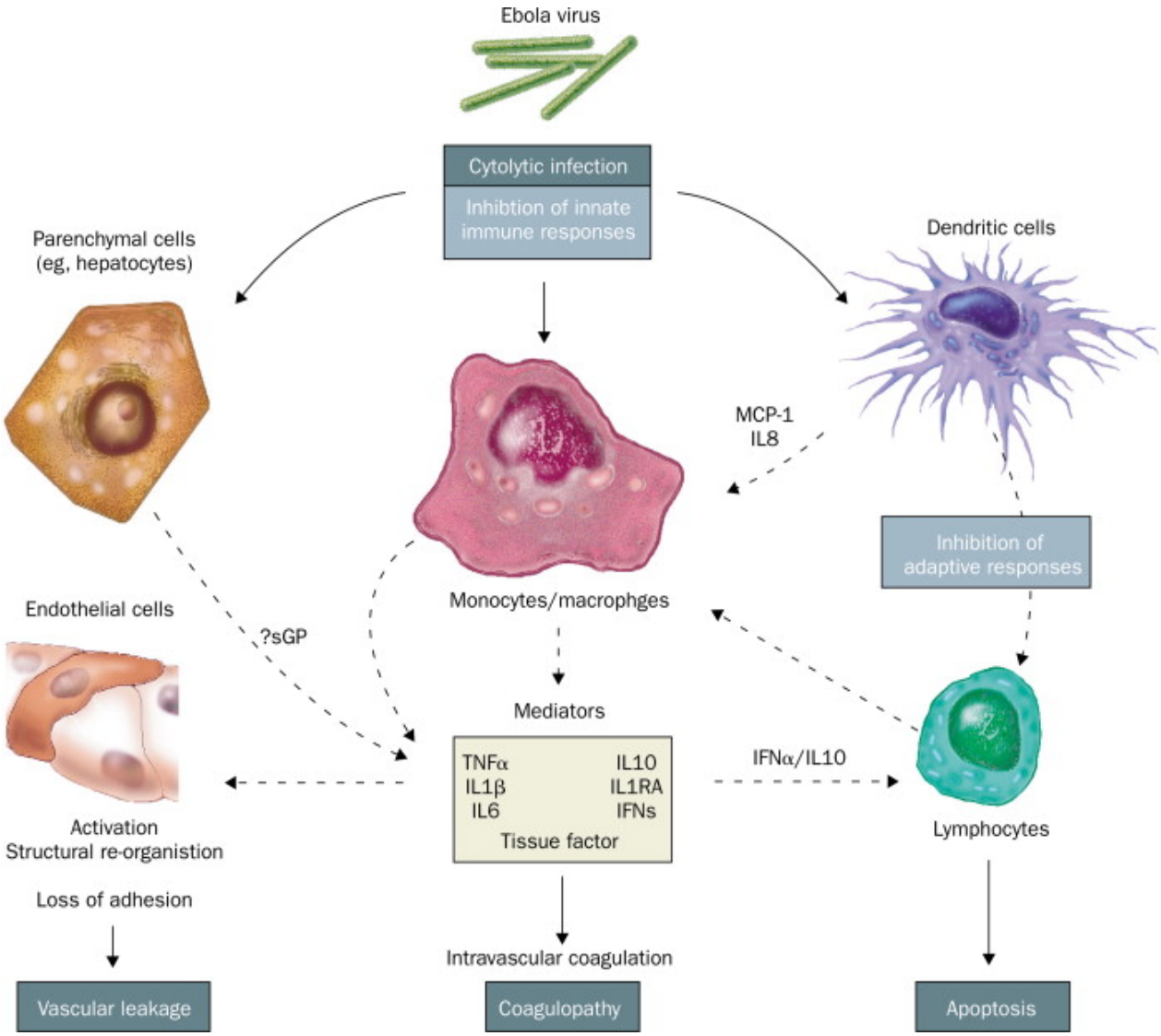
Pharmacotherapy of Ebola hemorrhagic fever

The mainstay of management of patients with Ebola disease is isolation of patients and use of strict barrier nursing procedures; the present treatment strategies are mainly symptomatic and supportive (fluid resuscitation, antypyretics, antidiarrheal drugs). Currently, there is no approved therapy for Ebola hemorrhagic fever (EHF), however several advanced treatment options were tested in animal models (on non-human primates or rodents). They include use of both symptomatic (e.g. use of tissue factor inhibitors - rhNAPc2, rhAPC - to abolish coagulopathy) and specific antiviral approaches: e.g. monoclonal anti EBOV antibodies (ZMapp, MB-003), phosphorodiamidate morpholino oligomers (PMOs), liposomes containing siRNA (LNP-siRNA:TKM-Ebola) and small molecule inhibitors (e.g. BCX4430, favipiravir). The scope of this article is to briefly review the most promising therapeutics for EHF, based on the data coming from rare clinical reports, studies on animals and results from in vitro models.[19]

New substituted benzimidazole derivatives

The benzimidazole nucleus is found in a variety of naturally occurring compounds and is of significant importance in medicinal chemistry. Owing to its conspicuous pharmacological properties, benzimidazoles have become an important pharmacophore and sub-structure in drug design and have been screened for a wide range of biological activities.

Benzimidazole derivatives are remarkably effective compounds to many diseases and may have the potential to be the first effective therapy against Ebola virus. Therefore, it is of great importance to develop specific design software and sustainable industrial synthetic routes for the development of effective and clinically relevant benzimidazole compounds.[18]



Pathogenesis of filoviral haemorrhagic fever

Ebola Virus Pathogenesis

Previous studies have shown that inoculation with EBOV is rapidly followed by viremia, with the virus spread throughout the host's tissue and organ systems.[20] Animal studies suggest that EBOV viremia starts from 2 to 4 days after inoculation.[21] An early target of the EBOV appears to be cells of the mononuclear phagocyte system —especially macrophages.[22] As a result, EBOV replication in the lymph nodes and spleen occurs early in the disease course.[23] Later stages of infection are characterized by EBOV replication in the interstitial fibroblasts of various tissues, including the lungs. More recent studies in the macaque animal model showed that dendritic cells are early and sustained targets for EBOV, and this target, in part, explains the immunosupression induced by EBOV.[24] In animal studies and human disease, disseminated intravascular coagulation has been observed.31 In addition, animal studies have shown that the EBOV transmembrane glycoprotein GP(1,2) and the EBOV matrix protein VP40 activate endothelial cells and induce a decrease in barrier function.[25] This viral effect likely contributes to hypotension and vascular instability. This decreased endothelial cell function appears to be compounded by activation of cytokine tumor necrosis factor-a, which is known to induce a long-lasting decrease in endothelial cell barrier function and is hypothesized to play a key role in EBOV pathogenesis.[26-27]

Current Status of Antiviral Compound Development for Ebola Virus Disease

In the past several years, a number of laboratories and industry-based researchers have used reverse genetics to identify new targets within viral genomes for drug and vaccine development. Reverse genetics allows development of recombinant filoviruses, such as EBOV, that contain key gene sequences but are nonreplicating and therefore noninfective. Reverse genetics has been applied in EBOV research to understand gene function as demonstrated in a study by Martinez and colleagues.[28] This team conducted studies of the EBOV VP30 and developed a model for VP30 phosphorylation that is dynamic and represents an important mechanism for regulation of the EBOV replication cycle. In another study by Blaney et al.,[29]reverse

genetics was applied to express a Zaire EBOV (ZEBOV) glycoprotein. This team created a rabies virus (RABV) vaccine that efficiently expresses ZEBOV glycoprotein and induces humoral immunity against both RABV and ZEBOV, while conferring protection against lethal RABV and EBOV challenge in mice. Reverse genetics has also enabled creation of virus-like particles (VLPs) that have morphology identical to actual EBOV but are nonpathogenic.[30] These VLPs, generated in a plasmid-based system, allow study of EBOV entry, replication, and assembly without the need for biosafety level 4 containment. Such a system has potential application in the development of EBOV vaccines. Finally, reverse genetic techniques for EBOV have allowed high-throughput screening to identify potential drug targets on the virus without requiring biosafety level 4 containment facilities.[31-32]

Immunotherapeutics

A novel immunotherapeutic, consisting of a combination of monoclonal antibodies (mAb),[33-34] has undergone success in animal studies[35] and is now undergoing further evaluation following its administration to approximately seven patients with EVD in the United States and other countries. Recently, the composition of the mAb combination (now referred to as ZMapp) was described.[36] The results of this study suggest that individually, the mAbs bind the EBOV glycoprotein core. ZMapp is currently manufactured in a tobacco plant–based production facility where plants are genetically modified to express monoclonal antibodies to EBOV glycoproteins.[37] The present formulation of ZMapp is a combination of two mAb cocktails referred to as Mb-003 and ZMab. Currently, ZMapp is in short supply, but plans are under way to scale up production to meet potential demand in the current West Africa EVD outbreak.

Progress of vaccine and drug development for Ebola preparedness

cAd3 Ebola Vaccine

After long time efforts related to develop vaccine platform using recombinant chimpanzee adenovirus vector for Ebola vaccine, Okairos (acquired by GSK) manufactured cAd3 Ebola vaccine (recombinant chimpanzee adenovirus serotype 3 vectored Ebola vaccine) and this candidate has been tested for clinical phase I by GSK.

Adenovirus vector has been considered as an effective platform for the DNA vaccine for a wide range of infectious pathogens such as HIV and tuberculosis, and the vaccine research center of NIH studied availability of cAd3 Ebola vaccine containing Ebola GP gene to be expressed in hosts after vaccination. Chimpanzee adenovirus vector seems to be safe because the animal vector do not replicate in human hosts and the cAd3 already showed hopeful result that protected all 16 animals from EHF after single vaccination.[38-39] There are two adenovirus vector-based Ebola vaccine candidates; monovalent vaccine against only Zaire strain, and bivalent vaccine against Zaire strain and Sudan strain. The monovalent vaccine is the candidate scheduled on September 2014 for the phase I clinical test in West Africa. Safety and immunogenicity of the cAd3 in human hosts will be announced in 2015.

Recombinant Vesicular Stomatitis Virus Ebola Vaccine

Recently, trial using vesicular stomatitis virus (VSV) platform designed as bivalent vaccine against Ebola and Marburg viruses revealed systemic immune responses protecting animals after injection.[40] This non-segmented, negative stranded RNA virus is also considered as a promising candidate for the recombinant DNA vaccine platform against many filoviruses because the virus is an animal pathogen which usually does not induce any severe symptoms in human,[41-42] and the most advanced form using VSV for Ebola vaccine is VSVΔG-ZEBOV vaccine developed and sponsored by the Public Health Agency of Canada and NewLink Genetics Corporation. The VSVΔG-ZEBOV vaccine contains highly attenuated recombinant VSV with substituted Ebola virus Zaire envelope GP and it can be cultured quickly for high titer. The VSVΔG-ZEBOV vaccine candidate also revealed the 100% protection efficacy in animals, furthermore this form of vaccine was administrated for a postexposure human patient injured by laboratory accident in German research group. This candidate is also to be conducted in African countries.

CONCLUSION

Even though the EVD is still in outbreak and keeps spreading in Africa, there are no effective vaccines to protect people or no approved therapeutics to rescue the infected patients either. It seems long way to stop the current outbreak in Africa, also to clearly extinguish the threatening generated by the highly fatal pathogens such as Ebola virus. However, saving stockpile of vaccines after quick process of development will be the most effective way to prepare the crises related to the biological agents. As a warning of EVD outbreak which has lethal fatality just after infection, we have to recognize the request for vaccine development with huge scope of potential pathogens which can be threatening in the future. Vaccination should be ultimate responsiveness against outbreak while the efficient therapeutics is urgently requested for the treatment of patients in the affected countries. It is the best strategy to quickly make current vaccine development successful to prevent the world from spread of Ebola virus.

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