CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Ebola outbreak in Western Africa 2014: what is going on with Ebola virus?

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The 2014 outbreak of Ebola virus disease (EVD) in West Africa, caused by Ebola virus (Zaire Ebola virus species), is the largest outbreak of EVD in history. It cause hemorrhagic fever in human and nonhuman primates with high mortality rate up to 90% and can be transmitted by direct contact with blood, body fluids, skin of EVD patients or persons who have died of EVD. As of December 17, 2014, 450 healthcare personnel are known to have been infected with Ebola, of whom 244 died. For development of Ebola vaccine and treatment are highly difficult due to its dangerous and accessibility that requires biosafety level 4 (BSL-4) to conduct experiment. Also there is no specific vaccine and treatment for Ebola virus; however, many candidate vaccines and antiviral-drugs such as ZMapp and TKM-Ebola are being developed for Ebola virus disease. In this review, we focus on the epidemiology of 2014 outbreak of Ebola virus and candidate agent for preventing and curing from Ebola virus.

Keywords: Ebolavirus, Vaccines, Epidemiology, Therapy

Introduction

Ebola viruses are the causative agents of Ebola hemorrhagic fever (EHF), which are highly virulent zoonosis that affect both human and nonhuman primates. Since then Ebola outbreaks have been reported on average every 1.5 years, with a total of 7 prior outbreaks generated over 100 reported cases. A recent study has estimated 22 million people distributed in areas of Central and West Africa to be at risk of Ebola. Ebola virus contains single-stranded negative RNA linear genome, about 18-19 kb in size and encode seven genes (NP, VP35, VP40, VP30, VP24, L, and GP) [1]. Five genetically distinct Ebola virus species within the genus Ebola virus are known (Zaire Ebola virus [ZEBOV], Sudan Ebola virus [SEBOV], Côte d'Ivoire Ebola virus, Bundibugyo Ebola virus [BEBOV], and Reston Ebola virus [REBOV]). The genomes of the five different Ebola viruses (BEBOV, ZEBOV, REBOV, SEBOV, and Taï Forest ebolavirus) are different in sequence and the number and location of gene overlaps. However, REBOV species is reported to cause disease only in nonhuman primates, ZEBOV, SEBOV, and BEBOV are responsible for most of the EHF outbreaks [2,3] but ZEBOV constitutes a particularly serious threat to both human and animals in sub-Saharan Africa with case fatality rates as high as 90%. The 2014 outbreak of EHF in West Africa, caused by ZEBOV is the largest outbreak of EHF in history. Fruit bats are believed to be the normal carrier in nature, although the means of local enzootic maintenance and transmission of the virus

within bat populations remain unknown. The virus is transmitted from wildlife to people through contact with infected fruit bats, and through intermediate hosts, such as monkeys, apes, or pigs that have themselves become infected through contact with bat saliva or faeces. So far there are no approved antiviral drugs or vaccines against Ebola viruses. The prevention of EHF requires improving our understanding of the epidemiology of the disease. In this review, we report important epidemiologic features related to Ebola outbreaks in Africa based on previous findings during major outbreaks that occurred on the continent. Also we elucidate a current status of promising Ebola vaccine and drug that is being developed.

Epidemiology

Contacting with the unknown reservoir host, Ebola virus has been circulated among wild nonhuman primates. Subsequent to outbreak of Ebola virus in wild environment, chimpanzee and gorilla population were remarkably reduced [4]. Also this have negatively affected to animal, source of food, which resulted in human epidemics.

Ebola virus is divided into five different species (the Zaire, Sudan, Ivory Coast, Bundibugyo, and Reston agents) and they are different in virulence to humans [5,6]. Zaire species, first recognized appearance in 1976, has overspread in a variety of regions with high mortality rate up to 88%. Sudan virus is related to approximate 50% case-fatality rate in four known epidemic cases [6]: two in Sudan in the 1970s, one in Uganda in 2000, and another in Sudan in 2004. Ivory Coast virus case for human has only been known in ethologist who conduct necropsy on a chimpanzee found dead in the Tai Forest where population of ape were remarkably decreased [3]. The Bundibugyo virus causing an outbreak of hemorrhagic fever with lower case-fatality rate (approximately 30%) than Zaire and Sudan viruses, are emerged in Uganda in 2007. Sequence analysis has revealed that the agent is most closely related to the Ivory Coast agent [7,8]. Reston virus is maintained in an animal reservoir in the Philippines, which has not been found in Africa. Therefore it differs from the others [8]. Ebola Reston virus was recognized first when it caused an outbreak of lethal infection in macaques imported into the United States in 1989 [9]. Nothing further was heard of the Reston virus until 2008, when the investigation of an outbreak of disease in pigs in the Philippines unexpectedly revealed that some of the sick animals were infected both by an arterivirus, porcine reproductive and respiratory disease virus, and by Ebola Reston virus.

However, most previous Ebola outbreak occurred in Central Africa, Ebola outbreak has started in the West African nation of Guinea which is confirmed by the World Health Organization (WHO) in late 2013. This outbreak was spread to Liberia, Sierra Leone, Nigeria, Senegal, and Mali [10]. Viral sequence of Ebola patients in Sierra Leone showed that the epidemic was originated from sustained person-to-person transmission without additional introductions from animal reservoirs. Its case-fatality rate has been estimated approximately 70% [11]. In Liberia and Sierra Leone, the magnitude of the outbreak was not clearly underestimated because of individuals with Ebola virus disease being cared for outside the hospital setting. Accumulative number of presumable, suspected, and laboratory-confirmed case of Ebola virus is 19,065 including 7,388 deaths as of December 17, 2014 (Table 1). These comprise 564 healthcare workers died approximately 50% [12]. WHO informed that Senegal and Nigeria became free from Ebola outbreaks by October 17 and 19, respectively due to the fact that no more case report of Ebola virus in both Senegal and Nigeria from September 5, 2014 and August 29, respectively.

There were reports of Ebola virus diseases case outside of West Africa [12]. Ebola virus diseases associated with Ebola outbreak occurred to healthcare workers who caring for patients suffered from Ebola virus disease, as well as a returning traveler. The index case of Ebola virus diseases associated with outbreak were reported in the Democratic Republic of Congo in August of 2014, which a pregnant woman is infect-

Table 1. Case count of Ebola outbreak 2014 (December 17, 2014)

	Total cases	Lab-confirmed cases	Total deaths	Country
Countries with widespread transmission	2,453	2,164	1,550	Guinea
	7,819	3,021	3,346	Liberia
	8,759	6,856	2,477	Sierra Leone
Initial case or cases and/or localized transmission	4	4	2	United States
	8	7	6	Mali
Previously affected countries	20	19	8	Nigeria

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ed from bushmeat which was killed by her family in wild. A total of 66 cases of Ebola virus diseases (confirmed and suspected), including 49 deaths are associated with Ebola outbreak as of November 9, 2014. Although there is no relation with the current epidemic in West Africa, sequence of Zaire strain of Ebola virus causing this outbreak is most closely related to the case that occurred in 1995 outbreak in Kikwit [13].

Diagnosis

It is difficult to diagnosing Ebola virus in advance from infected person owing to nonspecific symptoms which are often seen in patients who is suffering from more common diseases such as malaria and typhoid fever. After symptoms with high levels of circulating virus within the patient's body appear, seroconversion of Ebola virus diseases can be detected in blood. This require three days to reach for viral detectable levels. Laboratory test conducted in diagnosis such as antigen-capture enzyme-linked immunosorbent assay (ELISA) testing, IgM ELISA, and polymerase chain reaction (PCR) using specific primers are used within a few days after symptoms begin. Immunohistochemistry testing, PCR, and virus isolation for patients who is expired could be tested (Table 2).

Vaccines and Therapeutics

Many national departments of drug including WHO have researched useful treatments and vaccines, though there are no effective and target vaccine or treatment which are approved by Food and Drug Administration (FDA) applicable for human use. It is strongly required to focus on clinical management of additive care for complication such as hypovolemia, electrolyte abnormalities, hematologic abnormalities, refractory shock, hypoxia, hemorrhage, septic shock, multi-organ failure, and disseminated intravenous coagulation. For now, whole blood transfusions from convalescent patients are highly recommended for treatment of EHF. Although live attenuated vaccine and recombinant protein have been actively studied for Ebola virus based on murine model by 1990s, immunogenicity and the biosafety of those ways were not clearly described. DNA vaccine synthesizing immune-related genes in host cell or haring viral genes such as nucleoprotein (NP), glycoprotein (GP) in plasmid vector showed high efficacy to guinea pig and mouse model against various infectious diseases. While it was successful to induce immunization with DNA vaccine for Ebola virus in mouse model, DNA vaccine showed less effect on nonhuman primate and human models. Replacement of plasmid DNA vector to poxivirus, a viral vector carrying the genes of viral protein, lead out greatly increased antibody titer and cellular immunity. Also adenovirus which is impaired in replication for enhancing safety have been used as a priming agents, which show high cellular immune response and humoral immunity in cynomolgus macaques. The animal which is challenged by lethal viral dose, however, it showed complete protection, showing the way to prevent Ebola virus on primates against infection. In recent days, vesicular stomatitis virus (VSVΔG-ZEBOV) and chimpanzee adenovirus (cAd3-EBO Z) are being actively studied as a promising vaccine for Ebola virus diseases. VSVΔG-ZEBOV, a candidate vaccine for the Ebola filovirus, is a DNA vaccine that is developed by NewLink Genetics and Public Health Agency of Canada [14]. The vaccine consists genes for the surface protein of ZEBOV in attenuated vesicular stomatitis virus (VSV), which interfere DNA recombination. Since VSV is not critical to human, its biosafety and immune response are being estimated in clinical human trials (phase I).

In addition, ChAd3-EBOV, a candidate of Ebola virus vaccine, is a modified and attenuated chimpanzee adenovirus composed by GP of the Ebola virus [15]. It is developed by GlaxoSmithKline (GSK) and National Institute of Allergy and Infectious Diseases (NIAID). Although rescued virus could not easily replicate in humans, it stimulated a protective immune response in humans. NIAID and Oxford University have studied its effectiveness on clinical trials (phage I) for volunteer. Above this, MVA-BN, developed by Bavarian Nordic company, is a vaccine of attenuated vaccinia virus and AdVac, a DNA vaccine based on adenovirus, is being developed. And clinical trial will be conducted in 2015 years (Table 3).

Furthermore, as monoclonal antibodies, RNA-based drugs, and small antiviral molecules novel therapeutic drugs are ac-

Table 2. Current diagnosis of Ebola virus

Timeline of infection	Diagnostic tests available
Within a few days after symptoms begins	ELISA
	IgM ELISA
	PCR
	Virus isolation
Later in diseases course or after recovery	IgM and IgG antibodies
Retrospectively in deceased patients	Immunohistochemistry testing
	PCR
	Virus isolation

ELISA, antigen-capture enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

Table 3. List of Ebola virus vaccine that is actively being developed for clinical use

Vaccine	Status	Feature	Company
cAd3-ZEBOV	Phage I	Attenuated adenovirus	GSK and NIAID
VSVΔG-ZEBOV	Phage I	Attenuated VSV	NewLink Genetics and PHAC
MVA-BN	2015[a)]	Attenuated vaccinia virus	Bavarian Nordic
AdVac	2015[a)]	Attenuated adenovirus	CruceII
SynCon	Pre-clinical	Polyvalent vaccine	Inovio
VesiculoVax	Pre-clinical	Attenuated VSV	Profectus BioSciences

NIAID, National Institute of Allergy and Infectious Diseases; PHAC, Public Health Agency of Canada; VSV, vesicular stomatitis virus.
[a)]Scheduled for 2015 clinical trial.

Table 4. List of Ebola virus drug that is actively being developed for clinical use

Drug	Status	Feature	Company
ZMapp	Phage I	Three chimeric monoclonal antibodies	LeafBio, Inc.
Favipiravir	Approved for IAV	Inhibition of viral RNA-dependent RNA	Fujifilm
TKM-Ebola	Phage I	siRNA	Tekmira
Brincidofovir	Phage III	Oral nucleotide analog	Chimerix
BCX4430	pre-clinical	Inhibition of viral RNA polymerase	BioCryst
AVI-7537	Phage I	Binding Ebola RNA	Sarepta

IAV, influenza A virus.

tively being studied to be useful treatment. ZMapp, one of the powerful anti therapeutics, is an experimental biopharmaceutical drug with three chimeric monoclonal antibodies under development as a treatment for Ebola virus diseases [16]. The drug was first tested in humans during the 2014 West Africa Ebola virus outbreak, but has not been subjected to a randomized controlled trial to determine whether it works, and whether it is safe enough to allow on the market. It was first used experimentally to treat some people with Ebola virus disease during the 2014 West African Ebola outbreak, but as of August 2014 it had not yet been tested in a clinical trial to support widespread usage in humans. Although ZMapp use plant as a production unit, considering an innovative ways to produce antiviral drug, its productivity is too low to provide in time. Therefore, more study for producing ZMapp is needed.

Favipiravir, also known as T-705 or Avigan, is an experimental anti-viral drug being developed with activity against many RNA viruses: influenza viruses, West Nile virus, yellow fever virus, and foot-and-mouth disease virus. It is a pyrazinecarboxamide derivative as like some other experimental antiviral drugs (T-1105 and T-1106), The mechanism of its actions is related to the selective inhibition of viral RNA-dependent RNA polymerase while it does not inhibit RNA or DNA synthesis in mammalian cells [17]. In 2014, favipiravir was approved in Japan for stockpiling against influenza pandemics. The drug appears to be effective in a mouse model of Ebola virus disease, leading for clinical use of favipiravir as a Ebola treatment. TKM-Ebola, as known as Ebola-SNALP, which is a combination of Small interfering RNAs is being studied. It targets three proteins of Ebola virus: Zaire Ebola L polymerase, Zaire Ebola membrane-associated protein (VP24), and Zaire Ebola polymerase complex protein (VP35). Phase I clinical trial of TKM-Ebola was assessed for its safety in healthy people. The FDA put the trial on clinical hold in July 2014 to assess results, after some subjects had flu-like responses. In August, the FDA changed the status to "partial hold," allowing the drug to be used under expanded access in people infected with Ebola but with the phase I trial still suspended (Table 4).

Research Facility for Ebola

Ebola virus is dangerous and exotic agent that poses high individual risk of laboratory infections and hospital settings, which are frequently fatal because there are no vaccines or treatments. For these reasons, experiments with Ebola virus have to be performed in biosafety level 4 (BSL-4) laboratories. Currently, there are 21 BSL-4 facilities worldwide, however almost are operated in United States and Europe (Fig. 1). In East Asia, China and Japan are running the BSL-4 centers and developed Ebola treatments and detection technologies, while South Korea still has not only been equipped the facility yet but also scientists who are trained to work in BSL-4. It evokes urgent investment on facilities and training researchers for utilizing BSL-4 to keep up advanced defense for Ebola outbreak.

Conclusion

As the natural history and reservoir of Ebola viruses are not perfectly elucidated, there have been no specific methods for avoiding infection from the natural exposure. However, in these days, extensive studies performed to determine the natural reservoir of Ebola viruses have identified in common species of fruit bat (*Rousettus aegyptiacus*) as a potential and promising candidate. Since there have been no officially licensed vaccines or antiviral drugs for the treatment of Ebola

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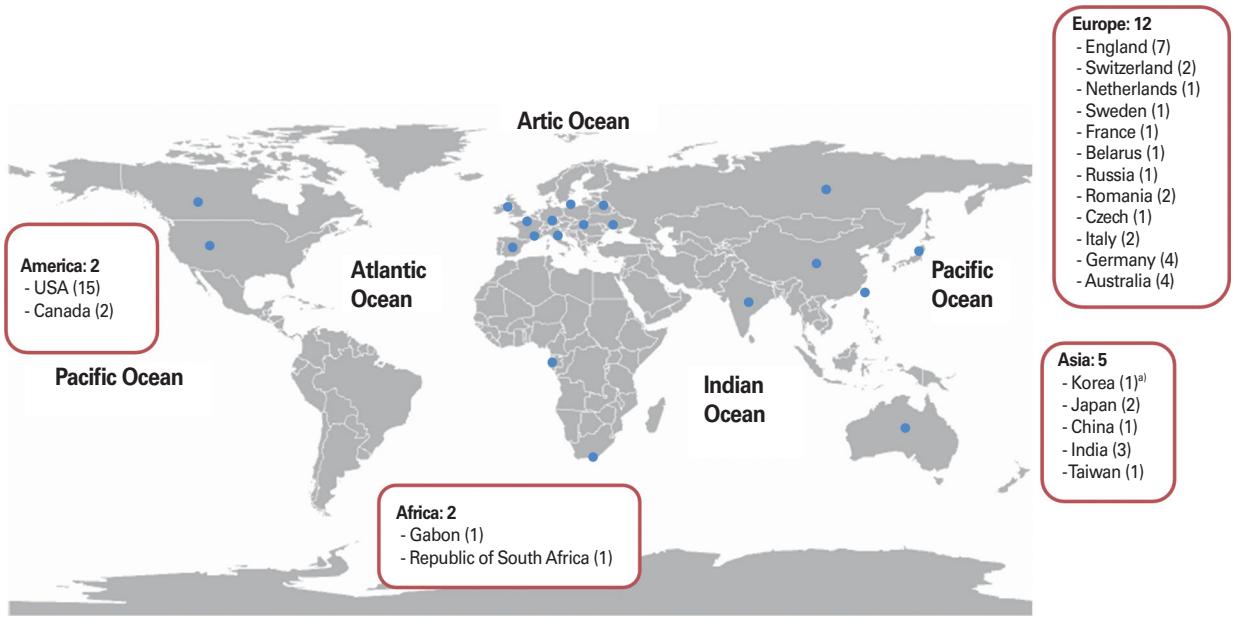

Fig. 1. Biosafety level 4 (BSL-4) facilities in the world. There are totally 54 BSL-4 facilities in the world: 15 in USA; 2 in Canada; 1 in Gabon; 1 in Republic of South Africa; 7 in England; 2 in Switzerland; 1 in Netherlands; 1 in Sweden; 1 in France; 1 in Belarus; 1 in Russia; 2 in Romania; 1 in Czech; 2 in Italy; 4 in Germany; 4 in Australia; 1 in Korea; 2 in Japan; 1 in China; 3 in India; 1 in Taiwan. [a]Under construction.

virus infections although there are effort to develop vaccine and treatment such as ZMapp and cAd-ZEBOV, early detection and diagnosis of infection from animals and human are very crucial for now. And for the prevention of transmission of the diseases, strict isolation of patient with fever and rigorous use of barrier and quarantine precautions are very important. Also, many experts and institution consider the Ebola virus as potential biological weapons [18]. Therefore, for the research of public health and biodefense against Ebola viruses, extensive studies of basic research including pathobiology, immune responses after infection should be intensively studied. In addition, additional BSL-4 containments which are only a few facilities exist worldwide are strongly required to study for preventing and treating Ebola virus diseases.

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