—Review—

Ebola Hemorrhagic Fever (EHF): Mechanism of Transmission and Pathogenicity

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Abstract

Hemorrhagic fevers represent a wide spectrum of viral infectious diseases, out-breaking mostly as epidemics, some of them being highly lethal. They range from those caused by bunyaviridae, associated with renal or pulmonary syndromes and those recently emerging and caused by the filoviridae family of thread-like viruses. Among the latter, Ebola hemorrhagic fever (EHF) bears the highest mortality and morbidity rates. One form of the disease has been documented only in monkeys. The human form, has occurred mainly in areas surrounding rain forests in central Africa. Patients present with signs of hemorrhagic diathesis, fever, diarrhea and neurological disorders, leading sometimes to confusion with local endemic diseases. Fatal victims of the disease die of dehydration. Poor hygienic conditions facilitate the spread of the virus. Biologically, the virus seems to target both the host blood coagulative and immune defense systems. Intensive epidemiologic search have failed to establish the definitive natural host of the virus. Twice, with a 19-year interval, major outbreaks have taken place in the Democratic Republic of the Congo. The second major outbreak in the northwestern city of Kikwit in April 1995 will serve here to elucidate the mechanism of the viral infection. (J Nippon Med Sch 2001; 68: 370—375)

Key words: Ebola, fever, transmission, pathogenicity, Congo

Introduction

Ebola hemorrhagic fever (EHF) has been reported as monkey infections in Italy (1992) and the USA (1990), but in humans, major outbreaks seem to have an ecosystem focusing on central Africa, which is surrounded by rainy forests extending from Gabon through the Democratic Republic of Congo (DRC) upward to Sudan. Isolated outbreaks have been reported in Ivory coast, also around rain forest areas. Although among the most profusely studied viral diseases of the last 2 decades, partly due to its dramatically publicized clinical presentation and a very short fatal outcome, one of its most vital epidemiological features, namely the natural reservoir, remains unknown. The disease affects only monkeys and humans. This evidence means that primates are highly suspicious natural reservoirs. Unfortunately, according to current knowledge, primates (humans as well as monkeys) seem to be mere intermediate or incidental reservoirs. Identification of the reservoir is a key to stopping the chain of disease transmission, and the only way to avoid a life threatening large scale outbreak. In the following pages, after a brief historical overview of outbreaks in Africa in general,

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we are going to show the chronological details of the most recent outbreak of EHF in DRC and to review what is known about the pathogenic mechanism of the disease.

I. Ebola hemorrhagic fever (EHF) as an entity

Ebola hemorrhagic fever (EHF) is a viral infectious disease caused by an RNA virus, phylogenically belonging to the filoviridae family of thread-like viruses, and is included in a group of recently defined emerging and reemerging hemorrhagic fever viruses. The virus has been named Ebola, after the name of the river that crosses the village of Yambuku, in northwestern Congo, where the first outbreak of the infection took place (**Fig. 1**). Recent studies seem to indicate the existence of viral strains selectively pathogenic for humans and animals, especially monkeys[1]. Infection, although occurring indirectly through body fluids, is strongly suspected to occur through airborne as well as skin contact transmission. There seems to be no gender predilection among the victims, but a focus on the 20-30-year-old age group has been observed. In the absence of drastic quarantine measures, the infection seems to be more severe and has a higher case fatality rate (>70%) than hemorrhagic fever caused by hantavirus in Korea (Seoul virus), in the Balkaans and in Scandinavia[2,3]. As of today, the disease has struck exclusively in Africa and 4 viral strains, respectively named Congo, Sudan, Gabon and Ivory coast after the African locations of successive outbreaks, are known as the causal agents of the human illness (**Table 1**).

II. General epidemiologic observations

As seen in the chronological review, outbreaks of Ebola hemorrhagic fever are relatively recent and clearcut clinico-epidemiologic features, allowing the establishment of the final diagnostic criteria are still elusive. To some extent, the last major outbreak in the district town of Kikwit, an agglomeration of 400,000 people, 200 miles away from the capital city of Kinshasa in DRC, was monitored in a way that helped outline parameters crucial to the understanding of some facets of the disease. That is why we will relate briefly the epidemiologic profile of this outbreak. As shown in Fig. 1, the town of Kikwit is located in the southeastern edge of the equatorial rain forest.

Clinical history in the Kikwit outbreak

The infection in Kikwit was essentially nosocomial and was introduced in the city's General Hospital on April 9, 1995, through a laparotomized paramedical staff menber who came from another hospital with a diagnosis of peritonitis. At the laparotomy, the diagnosis of peritonitis was excluded and an appendectomy was the main surgical act. Two days after surgery, signs of intra-abdominal bleeding indicated a second laparotomy that confirmed generalized organ bleeding, the first manifestation of a hemorrhagic diathesis. The patient died 3 days after this second laparotomy in an advanced stage of dehydration. Subsequently, the infection was spread by paramedical staffs who assisted in the 2 operations respectively, to their homes and to other hospitals where they served as ambulant helpers.

Epidemiological problems

A national committee on Health disasters, led by the Department of Microbiology of Kinshasa University, organized quarantine teams and took preventive measures, shutting down the city of Kikwit as well as all the hospitals but one, the General Hospital, where all the patients were gathered with a limited number of volunteer staff. A leaflet of information was conveyed to the general population. Within days, blood and stool cultures eliminated endemic enteritis, especially shigellosis and salmonellosis as causes of the disease. The results of blood samples sent to CDC/Atlanta confirmed Ebola virus as the causal agent. The epidemic, which was officially declared over 2 months later, claimed 244 deaths, among whom 60 were hospital staff.

Subjects learned from epidemiologic investigation

The transmission of the infection seemed very limited in hospitals with high hygienic standards. Even in the same hospital, the degree of protective garments (Surgical gloves, material for sterilization, etc.) were crucial to the containment of the viral spread, as testified to by the fact that the main surgeons of the index case operation have never been infected, for they probably used the few well preserved surgical gloves

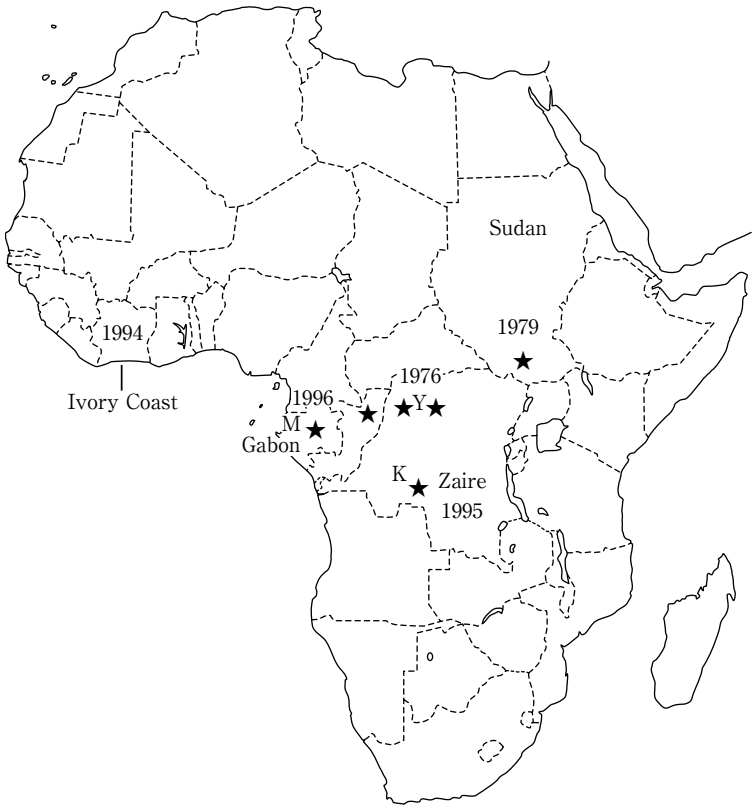

Fig. 1 Geographic representation of major Ebola hemorrhagic fever (EHF) outbreaks in Africa. In 1994 in Ivory coast among a group of wild chimpanzees and mainly in Gabon in 1996 (Maybout village: M), in Sudan in 1979 and twice in the Democratic Republic of Congo (Formerly Zaire on the map, respectively in the village of Yambuku, Y in 1976 and in the town of Kikwit, K in 1995). Stars indicate isolated outbreaks not cited in the legend.

Table 1 Casualties per year and per area of major Ebola Hemorrhagic Fever outbreaks in Africa

Country	Area	Year	Total number of cases	Number of death	Case fatality rate(%)
Congo (RDC)	Yambuku (North east)	1976	318	280	87
	Tandala (North east)	1977	1	1	
	Kikwit (Central east)	1995	315	244	77
Sudan	South west	1976	284	150	53
	South west	1979	34	22	64
Gabon	Mayibout (North east)	1996	37	21	57
	South west	1997	61	45	78

DRC : Democratic Republic of Congo
Source : Department of Microbiology, Kinshasa University, DRC
Le Gueno B. Lancet 1995；345：1271—1274

available in this countryside hospital. The main contamination pathway was through all kinds of body fluids (blood, urine, vomit, stools, etc.). Skin contact transmission as well as airborne transmission are highly suspected. Careful retrospective clinico-epidemiologic investigations suggested the existence of latent cases, isolated small outbreaks and cases that resolved unnoticed[4]. Also, a link could be established between the operated index case and a charcoal worker who died with all the signs of EHF 3 months prior to the big outbreak. This charcoal worker was working in the rain forest surrounding the Kikwit city area, where monkeys are hunted for business. Sometimes populations use monkeys for food in this area. But it has never been clearly established if and when the charcoal maker had dealt with these primates.

III. Pathogenic mechanism of ebola hemorrhagic fever

Virus as agent

A. Taxonomy and classification

Ebola virus is the second known filovirus in the world, the first one being the Marburg virus, which first erupted in 1967 and claimed the lives of 7 laboratory workers in Marburg (Germany)[5]. These viruses are classified as "**Filoviridae**", negative-strand RNA viruses. They are similar in morphology, density and polyacrylamide gel electrophoresis profile. Recent genome sequence data show them as being close to paramyxoviruses[6].

B. Virion composition

The virions of filovirus are pleomorphic by electron microscopy. The long filamentous form is the most common by appearing form, but "U", "b or 6" and circular forms are also demonstrated. Although a constant diameter of 80 nm has been common, the virions may vary in diameter up to 14,000 nm. The peak viral infectivity has been estimated to occur at 970 nm for the Ebola virus (790 nm for the Marburg virus) by rate zonal gradient centrifugation. Length is the unique differential feature known among members of the filovidae family[7]. The virion contains a nucleocapsid with a central space (20 nm in diameter) and a surrounding helical capsid (50 nm). The virions enter cells by endocytosis and replication takes place in the cytoplasm. During the phase of acute fever, the filoviruses can be isolated from the blood. These viruses grow easily in Vero cells.

C. Physical features

The viral particles have a molecular weight of $3-6 \times 10^8$ and a sedimentation coefficient of 13.000 to 14.000. Infectivity is stable at room temperature. The viruses are inactivated by UV, Gamma irradiation, 1% formaldehyde, beta propiolactone and brief exposure to phenol desinfectants and lipid solvents[8].

D. Genomic organization and properties

The nucleocapsid encloses a negative-strand RNA, without segmentation, which is complementary to the viral-specific messenger RNA. The genome of filovidae makes 1.1% of the total weight of the virion, with a length of 19 Kb[8]. The linear gene arrangement starting from a 3' end to a 5' end region, includes 7 bases in between. These genes are separated either by intergenic sequences or by gene overlaps. It has been confirmed that the Zairean viral subtype (this subtype should actually be called from now on Congolese subtype because of the country's name change in 1997) is the same virus as that from Yambuku (1976), Tandala (1977) and Kikwit (1995)[9]. The virus has demonstrated a high degree of genetic stability in nature. In the 19-year-interval between Yambuku and Kikwit, the virus has shown only 1.6% difference in nucleotide sequences. But between the Congo subtype and its Sudanese, Gabonese and Ivory coast homologues, up to 40% of nucleotide sequence difference has been observed[10]. These subtypes are distinguished by 4 genetic sequence arrangements of RNA, and the clinical severity of the disease depends upon these sequence arrangements. In the seven linearly arranged Ebola virus structural genes, the 4th protein gene (from the 3' end) encodes two glycoproteins, a virion surface glycoprotein (GP) that help the virus in the host cell binding, and a secretory glycoprotein (SGP)[10]. These 2 proteins seem to play a vital role in the pathogenicity of the infectious and hemorrhagic syndrome. The glycoprotein gene of all known Ebola viruses is encoded in 2 frames, which is one of the essential

genetic differences from the Marburg viruses. The Ebola and Marburg viruses are biosafety level 4 pathogens, to be dealt with only in maximum security laboratories.

Mechanism of infection

Experimental Ebola hemorrhagic-like fever has been induced in animals with no clearcut results. Both macroscopic and microscopic examinations of lesions induced by Ebola virus infection have been hampered by the paucity of necropsic materials[1]. Data available to date rely exclusively on the 5 necropsies realized in 1976 in the Yambuku outbreak. A ccording to current knowledge, 2 main alterations were observed during the syndrome[11]. The first is consistent with a hemorrhagic diathesis, involving all kinds of connective tissues but especially vascular connective tissue. Takada A et al[12], suggest that the surface glycoprotein (GP) of Ebola virus, in binding to endothelial cells to facilitate the replication of the virus, initiates a disseminated intra-vascular coagulation process that might explain the hemorrhagic diathesis. The DIC may then lead to the liver fibrinoid necrosis described by most investigators. The second alterations is massive damage to the cells of the phagocyte mononuclear system, which renders the host unable to generate an adequate immune response. Clinical findings indicated that during the active phase of the infection the patients are so vulnerable that the virus spreads through the body, crossing the blood-brain barrier without encountering any strong resistance. At the same time, most patients show a hematological profile characterized by profound neutro-lymphopenia. In this regard, the second viral glycoprotein (SGP) above cited, has been found to alter neutrophilic activation according to Yang and co-workers[12].

Insights in to the major unresolved issues and recommendations

Natural reservoir

Tremendous efforts have failed to determine the definitive natural host of the virus. Ksiazek[4] reported on investigations using as many as 30,000 different kinds of arthropods, rodents and primates (both humans and monkeys), without success. Prospective sero-epidemiologic studies as well as retrospective ones in areas of previous outbreaks, have been equally inconclusive. Despite these disappointing results, there is no doubt that the natural reservoir of Ebola viruses resides in these endemic regions. A number of predictable reasons may account for the lack of success in the identification of the virus's natural reservoir. Among them, seasonal variations deserve special attention. Availability of different animal species for blood sampling may be seriously influenced by seasonal variation. Finally, Johnson[4] made the hypothesis, awaiting verification, that Ebola virus might be a plant virus.

Ecology of EHF

Ebola hemorrhagic fever seems to appear in a repetitive fashion in tropical settings, with clinical manifestations closely resembling those of other local infections (salmonellosis, shigellosis, yellow fever, amibiasis, malaria, etc.). The poor health care system in those areas with inadequate disaster reporting strategies will still jeopardize the task of accurate and fast differential diagnosis and early detection of the outbreaks.

Hemorrhagic fevers, emerging and reemerging for two decades and caused by filoviruses or hantaviruses, are indeed humanitarian disasters that should be regarded as a world problem without boundaries and a concern for medical organizations all over the world. But since Ebola hemorrhagic fever, which seems to be the most virulent of these entities, has not outlined its ecology, it is first the duty of concerned nations to permanently monitor and forecast probable coming outbreaks. At first hand, the local doctors in concerned areas could be more useful than the well-equipped CDC doctors from Atlanta, if they could have the elementary tools to do the differential diagnosis of viral diseases. Almost 2 decades elapsed between the Yambuku outbreak and the Kikwit one, without due preparations that could have prevented the casualties of the latter outbreak. Better management of probable coming outbreaks and contribution to research concerning unresolved issues would necessitate the establishment of:

1. First aid laboratories in regions with rain forests with personnel trained for the fast detection of endemic infectious diseases and their differential diagno-

sis. These laboratories should be equipped with adequate biosafety systems, adequate communication equipment as well as bio-hazard material transportation channels.

2. A central institution for data centralization including epidemiologists, virologists, hematologists, pathologits and physicians. This institution, with the first aim of collecting data from different small units, will keep permanent contacts with laboratories collaborating in researches on the disease all over the world, but will also establish a unit of permanent education and training of medical and paramedical staff in high bio-safety level techniques, in patient handling, biological fluid and tissue sampling and also in anatomopathological procedures.

3. A team of epidemiologists with a background in quarantine methods for the purpose of preventive measures. Such a team will also include local guides to help researchers in their task of investigating the etiology concerning animals and plants of wild areas suspected to contain some possible natural viral hosts.

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