=== **REVIEWS** ===

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Ebola Hemorrhagic Fever: Properties of the Pathogen and Development of Vaccines and Chemotherapeutic Agents

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Abstract—Ebola hemorrhagic fever (EHF) epidemic currently ongoing in West Africa is not the first among numerous epidemics in the continent. Yet it seems to be the worst EHF epidemic outbreak caused by Ebola virus Zaire since 1976 as regards its extremely large scale and rapid spread in the population. Experiments to study the agent have continued for more than 20 years. The EHF virus has a relatively simple genome with seven genes and additional reading frame resulting from RNA editing. While being of a relatively low genetic capacity, the virus can be ranked as a standard for pathogenicity with the ability to evade the host immune response in uttermost perfection. The EHF virus has similarities with retroviruses, but belongs to (−)RNA viruses of a nonretroviral origin. Genetic elements of the virus, NIRV, were detected in animal and human genomes. EHF virus glycoprotein (GP) is a class I fusion protein and shows more similarities than distinctions in tertiary structure with SIV and HIV gp41 proteins and even influenza virus hemagglutinin. EHF is an unusual infectious disease, and studying the molecular basis of its pathogenesis may contribute to new findings in therapy of severe conditions leading to a fatal outcome.

*It's a pity that so much research
has the biological weapon aspect
in mind rather than helping
the affected population.*

Esther Sterk, Médecins Sans Frontières

INTRODUCTION

The first recorded outbreak of Ebola hemorrhagic fever (EHF) occurred in October 1976 in the Democratic Republic of Congo [1]. The outbreak of 2013–2014 is thought to share many features with the outbreak of 1976 [2], both being caused by the Zaire Ebola virus and starting in rural forested areas where hunting for bush meat is common [1]. Patients were admitted to regional hospitals in grave conditions with symptoms resembling malaria, typhoid fever, Lassa fever, yellow fever, or influenza [2, 3] The epidemics was a severe challenge for the health care systems of West African countries, and the total ill-preparedness of the community caused panic and social upheaval, requiring military units to bring the affected territories under control. Yet the most important lessons learned from the current situation with EHF are that health care systems are not prepared for such outbreaks in virtually all countries, vaccines are difficult to develop, and highly effective broad-spectrum antiviral chemotherapy agents are unavailable.

EBOLA HEMORRHAGIC FEVER VIRUS AND OUTBREAK OF 2013–2014

The virus *Zaire ebolavirus* is a causal agent of the EHF outbreak in West Africa. The Ebola virus was first identified in 1976 and caused sporadic EHF outbreaks with a high mortality in Africa, being assigned consequently to group A pathogens, which are potential bioterrorism agents [1, 4]. In terms of general virology, the Ebola virus is currently a reference standard of pathogenicity, and its studies are of basic importance for solving the most complex problems in treating and preventing virus infections.

The species *Zaire ebolavirus* is the most pathogenic species of the genus *Ebolavirus* (family Filoviridae, order Mononegavirales). The family Filoviridae

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Abbreviations: EHF, Ebola hemorrhagic fever; DIC, disseminated intravascular coagulation.

Order Mononegavirales
Family Filoviridae
Genus *Ebolavirus*
Species *Taï Forest ebolavirus*
Virus: Tai Forest virus (TAFV)
Species *Reston ebolavirus*
Virus: Reston virus (RESTV)
Species *Sudan ebolavirus*
Virus: Sudan virus (SUDV)
Species *Zaire ebolavirus*
Virus: Ebola virus (EBOV)
Species *Bundibugyo ebolavirus*
Virus: Bundibugyo virus (BDBV)

Fig. 1. Classification of viruses of the genus *Ebolavirus*. The virus responsible for the EHF outbreak of 2014 in West Africa is in bold [1, 2, 6].

includes three genera: *Ebolavirus, Marburgvirus*, and *Cuevavirus*. *Ebolavirus* species are morphologically similar to *Marburgvirus* species, but have different antigens. The genus *Ebolavirus* includes five species: *Sudan ebolavirus*, *Zaire ebolavirus*, *Taï Forest ebolavirus*, *Reston ebolavirus*, and *Bundibugyo ebolavirus*. Infection with *Reston ebolavirus* is asymptomatic, while the other four species are highly pathogenic for humans [1–5]. The taxonomy of the genus *Ebolavirus* has been considered in a special article published in *Viruses* [6] by a team including Russian researchers who contributed substantially to studying the problem both in Russia and abroad. A current classification of the genus *Ebolavirus* is shown in Fig. 1.

The Ebola virus is found in primates, field mice, and bats. In bats, infection does not lead to a disease outbreak and is virtually asymptomatic. The fact suggests that bats, in particular, fruit bats of the family Pteropodidae, are a natural reservoir and intermediate host of the Ebola virus [7]. A genetic analysis of Ebola virus isolates showed that the current outbreak started with virus transmission from the straw-colored fruit bat *Eidolon helvum* to humans [2]. It should be noted that a greater range of intermediate hosts is possible for the virus.

It is of interest that viruses of the family Filoviridae belong to a limited group of viruses, known as the non-retroviral integrated RNA viruses (NIRVs), whose specific elements were identified in animal genomes [8]. A high structural similarity was observed for the receptor protein GP of the Ebola virus with the Env proteins of endogenous retroviruses and placental syncytins of humans [9]. Common elements of the Ebola virus and retrovirus are of principal importance for understanding the key factors of the Ebola virus pathogenicity [10, 11]. A high similarity was additionally detected between Ebola virus GP and well-known influenza virus hemagglutinin, facilitating a better understanding of the GP tertiary structure and inhibitor design [12, 13].

EPIDEMIOLOGY OF EBOLA HEMORRHAGIC FEVER

A team of WHO epidemiologists identified the Ebola virus as a causal agent of the hemorrhagic fever outbreak that occurred in the Ebola River valley (Zaire, Central Africa) in 1976 [1–3]. A total of 23 EHF outbreaks were registered since that time. The outbreak of 2014 started in Guinea (West Africa) and spread to Liberia, Sierra Leone, and Nigeria. A phylogenetic analysis of Ebola virus genomic sequences gives grounds to think that the virus strain responsible for the 2014 outbreak relatively recently found its way from Central to West Africa [2, 3].

A phylogenetic analysis confirmed genetic similarity between Ebola virus lineages involved in the three last EHF outbreaks. The finding suggests a common ancestor for the viruses involved and supports the hypothesis that each EHF outbreak was caused by independent transmission of the virus from one genetically diverse population [2]. The genetic similarity and close phylogenetic relationship of the genomes of 2014 Ebola viruses indicate that a single act of transmission from a natural reservoir was followed by stable transmission of infection among humans, leading to an epidemic outbreak. This epidemiological situation basically differs, for instance, from epidemics of avian flu, which was characterized by multiple focal outbreaks in endemic regions and a low transmission rate among humans. Hence, Ebola virus infection seems to be extremely contagious. There are estimates that a single event of crossing the species barrier would be enough for the virus to spread rapidly in the population [2].

The EHF outbreak starting in December 2013 involved mostly Guinea, Liberia, Senegal, Sierra Leone, and Nigeria among African countries [14–16]. Cases observed in the United States and European countries occurred in healthcare workers and missionaries. Estimates published in mass media for the risk of EHF being transferred to other countries, including Russia, are based on the passenger traffic statistics and
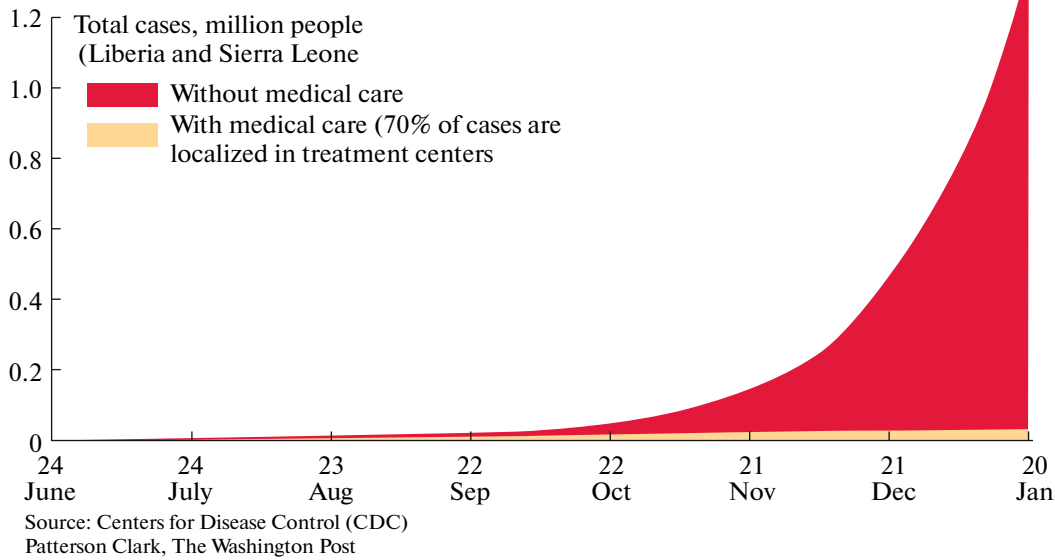
(a)
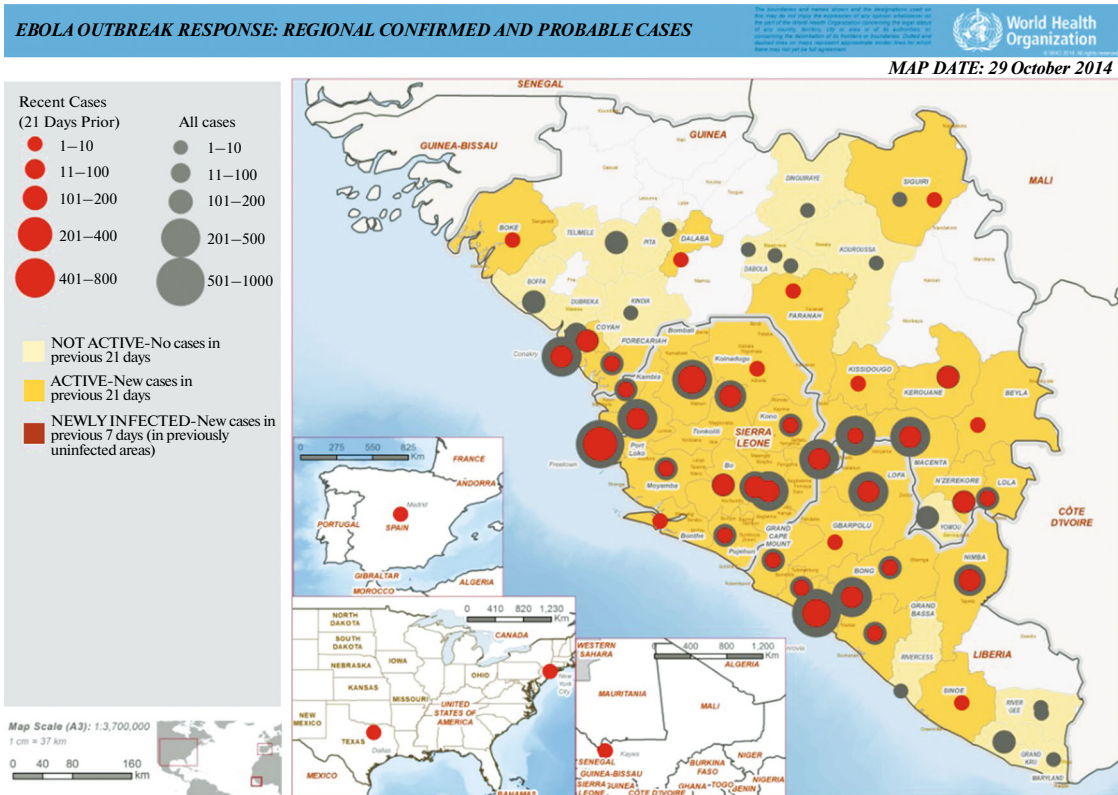
(b)

Fig. 2. EHF geographic distribution and morbidity in West African regions. (a) Distribution of EHF in West Africa with the number of cases shown graphically (WHO data as of October 25, 2014). (b) Forecast of EHF morbidity up to January 20, 2014 (data of CDC, Atlanta, United States). The number of cases is predicted to reach 1 million or even more people.

the number of flights from affected African countries. It is clear that the EHF spreading rate depends primarily on how stringent quarantine policy is in primary foci and how efficient is passenger monitoring. How-

ever, epidemiological studies show that these activities bring only a temporal success in the case of highly contagious infections with a prolonged incubation period.
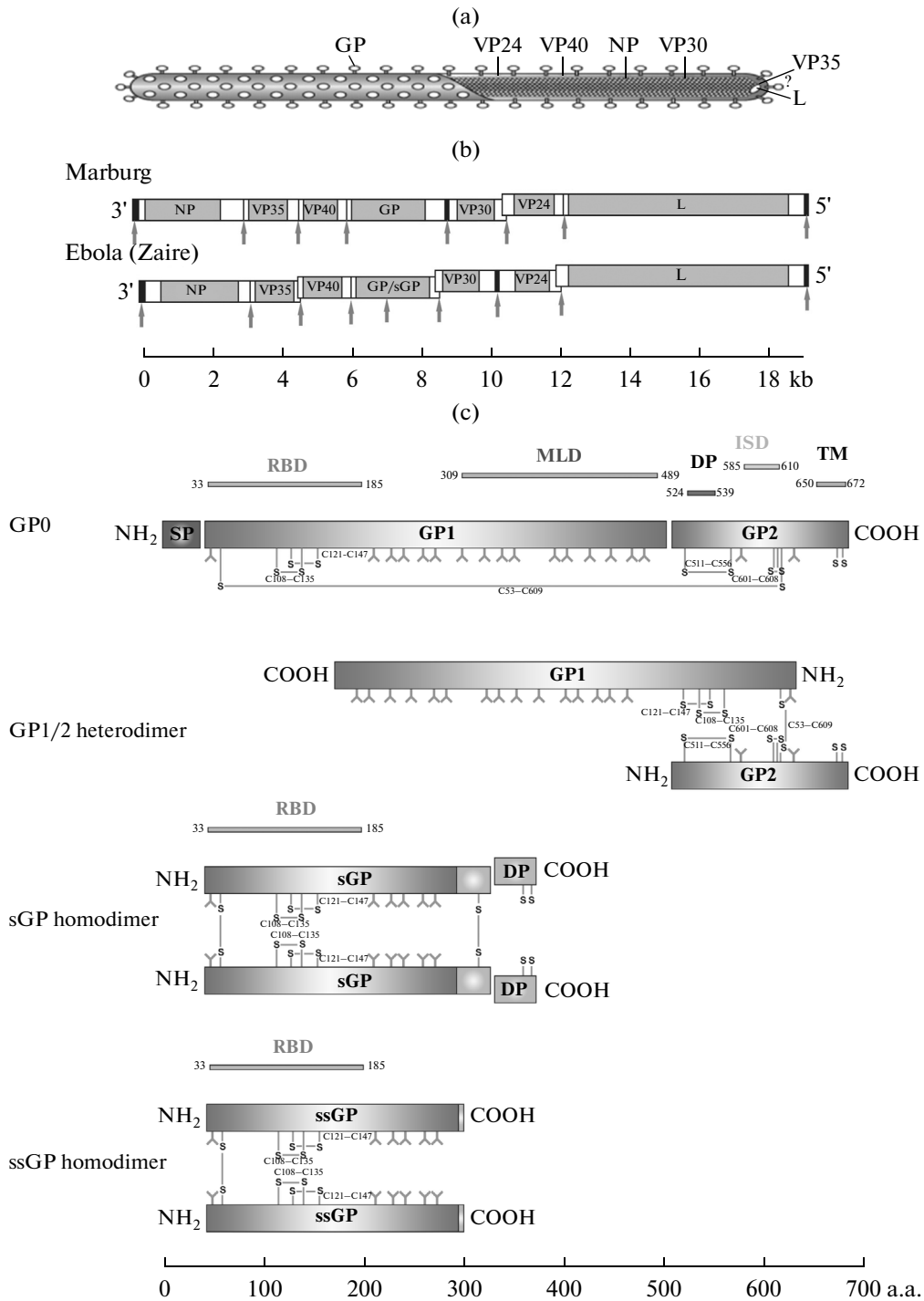
(a)

(b)

Marburg

Ebola (Zaire)

0   2   4   6   8   10   12   14   16   18 kb

(c)

GP0

GP1/2 heterodimer

sGP homodimer

ssGP homodimer

0   100   200   300   400   500   600   700 a.a.

Fig. 3. Scheme of the *Ebolavirus* virion and linear genome maps of the Marburg and Ebola (Zair) viruses. (a) Structure of the Ebola virus and localization of virus proteins. (b) Linear genome maps of the Marburg and Ebola viruses. The site of RNA editing and sGP synthesis initiation in the central region of the *GP/sGP* gene is indicated with an arrow. The site is absent from the Marburg virus genome. Intergenic untranslated regions are indicated with arrows along the map. (c) Physical map of GP (GP1/GP2). The C53−C609 disulfide bond (GP1−GP2) and internal cysteine loops are indicated. The coordinates are shown for the most important functional domains: RBD, receptor-binding domain; ISD, immunosuppressive domain; MLD, mucin-like domain; FD, fusion domain; TM, transmembrane domain; SP, signal peptide; and DP, δ-peptide.

Table 1. Ebola virus proteins and their functions and interactions with the cell [2, 13, 17, 18]

Protein	Function (effect on cells and organs)
NP	Nucleoprotein, a structural element of the virus nucleocapsid, plays a role in genomic RNA packaging into virions
VP35	Component of the polymerase complex, a main virulence factor, an interferon antagonist (along with GPs)
VP40	Matrix protein, plays a role in virion assembly and budding
GP	Surface glycoprotein; is responsible for receptor binding, virus penetration into the cell, and membrane fusion; is synthesized as a precursor and then cleaved into GP1 and GP2 by the protease furin
sGP	GP isoform resulting from RNA editing, is secreted in great amounts by infected cells and circulates in the peripheral blood, possesses immunosuppressive properties, and binds with virus-neutralizing antibodies to ensure virus evasion of the immune response
VP30	Minor nucleoprotein, is associated with the matrix protein, plays a role in virus RNA encapsidation, acts as a virus-specific transcriptional activator and a factor in reinitiating virus RNA synthesis
VP24	Minor matrix protein, is involved in virion assembly, was identified as an antagonist of signaling systems that activate antivirus protection (VP24 binds with a unique noncanonical nuclear localization signal of the cell protein karyopherin $\alpha5$, competing with phosphorylated STAT1, which is a main transcriptional regulator that activates expression of interferon-inducible genes)
L	Catalytic subunit of virus RNA-dependent RNA polymerase and nucleocapsid complex, consisting of L and VP53; has a unique capability of catalyzing RNA editing

The epidemiological situation as of late October 2014 and a forecast for a subsequent period (early 2015) are shown in Fig. 2 [14, 15]. The predicted numbers of cases of and deaths from Ebola change rapidly; the number of deaths may reach one million people in the nearest future according to some estimates. Moderate forecasts estimate the number of cases in the primary foci of the EHR outbreak at several tens of thousands of people. As of October 25, 2014, the Ebola morbidity rate ceased to grow in Nigeria. This is an early sign that the spread of infection can be controlled using anti-epidemic measures [15].

GENOME AND PROTEOME OF THE EBOLA VIRUS

The Ebola virus is a (−)RNA virus; i.e., its reproduction depends on its own polymerase complex. The Ebola virus genome is a single-stranded (−)RNA of approximately 19000 nt [19]. Seven open reading frames identified in the genome code for major virus proteins and occur in the following order: 3'-NP-VP35-VP40-GP/sGP-VP30-VP24-L-5' (Fig. 3). The genes are separated by short untranslated sequences [2, 13, 18]. The leader and trailer regions are not transcribed, harboring control signals for transcription, replication, and genomic RNA packaging in virus particles [18].

Functions of the virus proteins are summarized in Table 1.

The Ebola virus genes each code for one protein [13, 18]. The only exception is *GP*, which produces not only the main translation product GP, but also sGP, resulting from transcriptional RNA editing [13,

17, 18]. The 300 first amino acid residues of sGP are the same as in the N-terminal region of GP, but the C-terminal sequence of sGP is unique [18, 19]. Infected cells secrete sGP as a homodimer stabilized by disulfide bonds. sGP is an important component of the system that helps the Ebola virus to evade the immune response; its content in the peripheral blood increases in the course of infection. Shortened sGP (ssGP) was identified recently [13, 17−19].

Among all Ebola virus proteins, GP was studied most comprehensively (Fig. 3), and its spatial structure is known [17, 18]. GP attracts particular interest because it executes the most important function in the virus life cycle, interacting with a cell receptor and ensuring virus fusion with the membrane [18]. Cell infection starts with GP binding with the TIM-1 cell protein, which belongs to a class of receptors that possess T-cell immunoglobulin-like and mucin-like domains [20]. TIM-family proteins play a key role in regulating the cell immune response to virus infection. The TIM-1 receptor is involved in the allergic response and the pathogenesis of asthma [20]. The Ebola virus enters cells via clathrin-mediated endocytosis or receptor-mediated macropinocytosis [19−21]. The interaction of GP1 with the Niemann−Pick C1 cholesterol transporter (NPC1) was also found to play a role in virus entry into the cell at the late endosome stage [17]. In acidosis, highly glycosylated at the mucin-like domain, nonprocessed GP is cleaved in endosomes into GP1 and GP2 by cellular furin and the cysteine proteases CatL and CatB [21]. Proteolysis occurs during sGP formation as well. GP proteolytic processing leads to conformational changes in the GP2 trimer, which initiates fusion of the endosomal

Table 2. Clinical signs and symptoms of EHF*

Disease stage/phase	Time from first manifestation, days	Clinical signs and symptoms
Early fever stage	0—3	Fever, weakness, muscle aches
Gastrointestinal stage	3—10	Epigastric pain, nausea, vomiting, diarrhea Continuing symptoms: persistent fever, asthenia, headache, conjunctival infections, retrosternal pain, abdominal pain, joint and muscle aches, singulation, hiccups, delirium
Shock or recovery stage	7—12	Shock: impaired consciousness, coma, fast pulse, oliguria, anuria, tachypnea Recovery: gastrointestinal symptoms resolve, digestive tract functions normalize, appetite develops, and locomotor activity increases
Late sequel stage	≥10	Gastrointestinal bleeding, secondary infections, meningoencephalitis, marked neurocognitive impairment

* The fever stage of EHF corresponds to the early symptomatic stage; the gastrointestinal stage is a transition to the late symptomatic stage. The stage of shock (hypovolemic or septic) or recovery are terminal stages where severe conditions can be corrected therapeutically. The late sequel stage is a terminal stage with irreversible and fatal impairments in the functions of vital organs.

and virus membranes to release the virus nucleocapsid into the cytoplasm. It should be noted that GP contains virus-neutralizing antibody determinants and is consequently most often used to design anti-EHF vaccines [17, 21].

Protection from the host immune system involves several Ebola virus proteins (Table 1). In particular, VP35 and VP24 suppress innate immunity; VP35 inhibits interferon production; and VP24 prevents the transport of phosphorylated STAT1 into the nucleus, thus suppressing the interferon-mediated antivirus response [13, 17, 22—24]. There are many molecular targets of the Ebola virus among components of the host immune system. Their list can be extended to all levels of body protection from virus infections [22]. In general, the Ebola virus acts as the most potent immunosuppressive agent that causes immunoparalysis, rendering the organism incapable of recognizing virus antigens [10, 11, 24]. With a rapid development of infection, the multilevel immunosuppressive system prevents the body from producing an adequate immune response. This circumstance is to a great extent responsible for numerous deaths from EHF, which occurs as early as 9—12 days of disease. Other severe conditions include systemic organ failure, hemorrhagic pulmonary edema, sepsis, and disseminated intravascular coagulation (DIC) [25, 26].

CLINICAL PRESENTATION OF EBOLA HEMORRHAGIC FEVER

The EHF development includes the following stages: an incubation period, an early symptomatic stage, a late symptomatic stage, and a terminal or recovery period [16, 27]. Another classification in broad use is based on the consequent development of signs and symptoms of vital organ involvement (Table 2).

In total, the main clinical signs and symptoms of EHF resemble sepsis [25, 27]. The clinical status of EHF patients is characterized by systemic virus replication (high-level viremia), cytokine storm, general immune suppression resembling immunoparalysis, septic condition (an impaired function of the intestinal and vascular epithelia), impaired coagulation, tissue edema, and increased total vascular permeability. Patients die of multiple organ failure, acute respiratory distress, or coagulopathy [14, 16, 17]. The development of the infection process caused by the Ebola virus and the main components of its pathogenesis are shown in Fig. 4.

The Ebola virus infects immune cells [16, 28]. The most important consequences of infection are leukopenia, dissemination of virus infection in the body, and suppressed presentation of virus antigens by antigen-presenting cells (dysfunction and apoptosis of dendritic cells) [28]. Infection of endothelial cells alters the barrier function of the vascular endothelium and leads to hypovolemic shock and hemorrhagic tissue edema. Activation of tissue factors (plasminogen) leads to fatal coagulopathy. The immune response is suppressed at many levels, which is related primarily to the effect of the immunosuppressive domain of GP [10, 11].

Cytokine storm is an overproduction of pro-inflammatory cytokines, which cause a systemic inflammation syndrome with a diffuse inflammatory reaction of tissues and blood vessels. The EHF sequels develop in the presence of a cytokine storm and immunoparalisis due to infection of macrophages and dendritic cells and
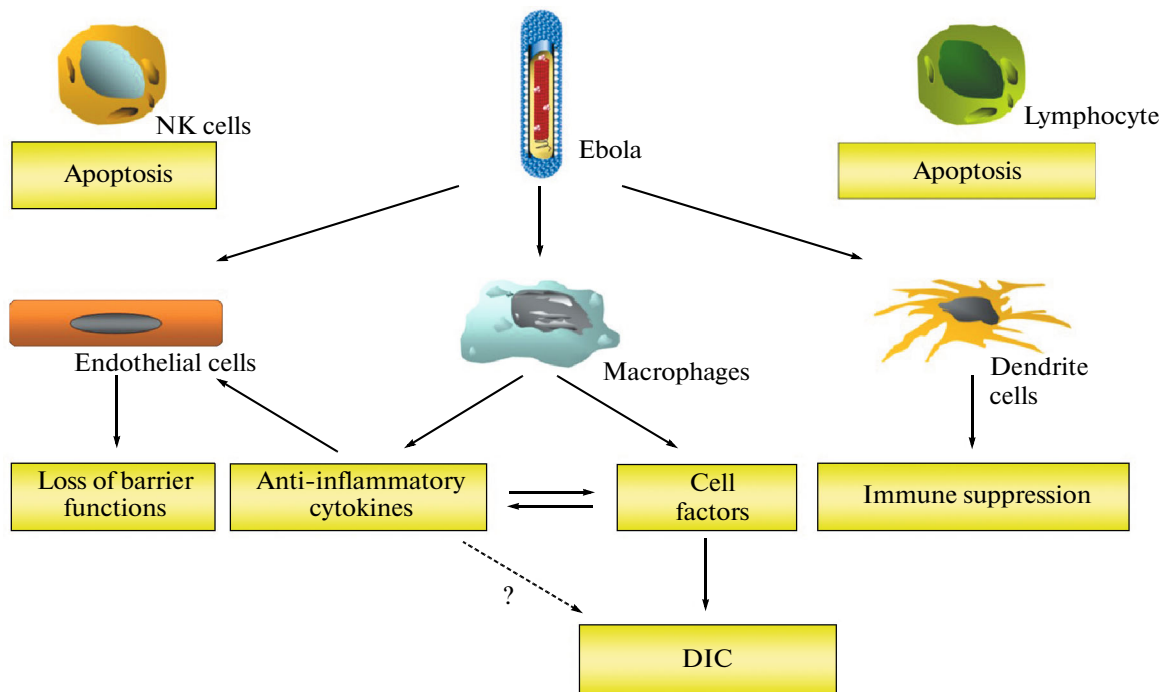
Fig. 4. Interaction of the Ebola virus with immune cells and the pathogenesis of infection.

a block of synthesis and functional activity of interferons [23–26].

The Ebola virus came into focus of relevant studies [22, 23] soon after a discovery of virus proteins acting as interferon antagonists [29]. As a result, VP35 was identified as a protein that to blocks interferon production in early infection [22, 23]. VP35 is generally thought to play a crucial role in the pathogenesis of EHF, contributing to the suppression of antivirus cell defense.

Oxidative stress. Endothelial cell infection and induced synthesis of enzymes that cause oxidative stress and mass apoptosis are of utmost importance for the pathogenesis of Ebola virus infection [26, 30, 31]. Infection of endothelial cells alters their intercellular contacts and the association with the intima (vascular basement membrane) of vessels. NO synthase expression is simultaneously induced in endothelial cells, resulting in NO overproduction [26] and nitrosative stress caused by peroxynitrite and other reactive oxygen species. A higher NO level in the peripheral blood correlates with the disease severity and high mortality [26, 30]. Erythrocyte hemolysis in tissues affected by hemorrhagic edema leads to the most destructive generation of reactive oxygen species, especially in the lungs [16, 32, 33].

Endothelial dysfunction and mass endotheliocyte apoptosis. Virus particle budding in lipid rafts of endothelial cells is thought to destabilize the vascular wall and thereby cause bleedings and hemorrhages. A direct effect of virus proteins on the endothelial barrier integrity cannon also be excluded [31–35]. A no less destructive effect is exerted by the tumor necrosis factor (TNF) as a leading component of the cytokine storm [25–27]. It should be noted that defects in the endothelial barrier arise primarily because cell contacts between endotheliocytes are structurally impaired [17, 26, 28, 31, 33–35]. Hence, therapy should be aimed at restoring the integrity of the endothelial barrier and protecting the endothelium from cytokine storm components [35–42].

Coagulopathy. Viremia rapidly develops in EHF and is accompanied by infection of vascular endothelial cells in early infection. A destructive effect of infection on the vascular endothelium and macrophage activation (Fig. 4) lead to disruption of intercellular contacts, causing endothelial dysfunction and consequent hemorrhagic tissue edema. Blood clotting accompanies hemorrhagies [17, 26, 28, 31, 33–35]. Activation of the tissue plasminogen activator develops as an inadequate compensatory reaction [31, 33–35], inevitably stimulating the coagulation cascade (Fig. 4). Disseminated intravascular clotting (DIC) is the most severe consequence of the process. The risk of DIC is substantially reduced by the use of activated protein C drugs (Xigris) [40, 41]. Treatment with recombinant nematode anticoagulation protein c2 (rNAPc2) was found to protect up to 33% of primates from fatal Ebola virus infection [42]. Large-scale generation of immune complexes in circulation is also considered to be a factor that provokes blood clotting [28, 43–45].

Table 3. Laboratory diagnosis of EHF

Disease stage	Diagnostic target	Diagnostic test	Material to be tested
Early symptomatic stage	Virus antigens, virus RNA Virus particles	ELISA and ICA for virus antigens, ICA for IgM, PCR Electron microscopy, virus isolation in cell cultures and laboratory animals	Blood, serum, tissues
Late symptomatic stage	Virus-specific IgM and IgG	ELISA for virus-specific IgM and IgG	Blood, serum
Recovery	Virus antigens, virus RNA*	ELISA for virus antigens (including assays for the sGP level in peripheral blood) and PCR to estimate the viral load	Blood, secretions, bed-clothes
Postmortem analysis	Virus antigens, RNA, virus particles	Virus isolation, PCR, immunohistochemistry	Tissues, clothing

The disease outcome is usually determined on days 6−11, when either the acute-phase crisis resolves or a transition to the terminal stage occurs [14, 29, 31−39]. This circumstance shows that the therapy window is limited and that a medical decision is to be made within a short while; it is therefore of immense importance to detect the turning period [25, 40−42, 46].

SPECIFICS OF THE EBOLA VIRUS INTERACTIONS WITH THE IMMUNE SYSTEM

The interaction of the Ebola virus with immune and other cells is illustrated in Fig. 4. The virus initially interacts with immune cells to cause the following alterations: NK cell apoptosis, cooperative lymphocyte apoptosis, dendrite cell infection, and macrophage activation with oxidative stress, which is associated with overproduction of reactive oxygen species.

Immunosuppression targeting NK and T cells is an important effect of infection [10, 11]. GP and the interferon antagonists VP35 and VP24 were identified as immunosuppressive virus proteins [22, 30, 47]. Higher pathogenicity of the Ebola virus compared with the Marburg virus is due to sGP synthesis [17, 24]. Antigenic subversion due to secretion of sGP as a truncated GP form [24] leads to antibody neutralization in circulation, thereby substantially reducing the immune response to infection. Thus, the Ebola virus targets various elements of the host immune system, such as monocytes, macrophages, and dendrite cells. Multiple organ failure is caused by infection and mass apoptosis of vascular and lung endothelial cells. Antibody-dependent enhancement (ADE) of the infection process is another mechanism involved in both stimulating the virus infection and facilitating host immunity evasion [39, 43, 44]. The virus capability of ADE should be considered when constructing vaccines [24, 43−45]. It is known also that the Ebola virus interacts with complement components and cell Fc

receptors to promote a spreading of infection among immune cells and various organs and tissues [22, 43].

DIAGNOSIS

A diagnostic monitoring is essential when a new infection arises or a known one recurs. Rapid laboratory diagnosis is one of the main prerequisites to a reliable infection control, timely identification of infected individuals, quarantine activities, and, most important, timely medical decisions in administering effective treatment in a timely manner. The main tests employed in laboratory diagnosis of EHF are summarized in Table 3. A RT-PCR test (Central Institute of Epidemiology, Russian Federal Service for Surveillance in Consumer Right Protection and Human Well-being) is currently approved in the Russian Federation.

Simple rapid tests are essential for diagnosis in the primary foci of infection [46, 47]. Rapid progress was seen again in developing rapid diagnostic tests on the basis of biochips, immunochromatographic strips, and various modifications of ELISA [46−48]. New approaches were proposed in several Russian projects.

DEVELOPMENT OF TREATMENTS FOR EBOLA HEMORRHAGIC FEVER: PRACTICAL REQUIREMENTS AND PRIORITY OF ANTIVIRUS DRUGS

Only limited agents effective in EHF are available for obvious reasons. Hence, the WHO had to intervene in the drug approval system and to support the vaccines and antivirus drugs that had been studied most comprehensively and tested in primates. A general opinion is that monotherapy is not promising in EHF [3, 4, 25, 28]. A combination of medications with intensive care is the only means to achieve a desirable effect [25, 28]. The efficacy of this approach can be

Table 4. WHO-supported vaccines and drugs for the prevention and treatment of EHF [49, 50]

Product	Nature	Efficacy and limitations of use	Reference
Vesicular stomatitis virus-based vaccine	Replication-deficient vector expressing GP	100% protection. Phase I clinical studies. **Should be stored at −70°C**	[51−55]
Adenovirus vector Ad5 (ChiAd3)-based vaccines	Adenovirus vector-based vaccines	High antigen loading or repeated vaccination is required to overcome pre-existing immunity. Chimpanzee adenovirus Ad3 shows better characteristics and provides an alternative to Ad5 vectors. Phase I clinical trials. **Should be stored at −70°C**	[52, 53, 55, 56]
ZMapp, a cocktail of humanized antibodies produced in *Nicotiana* plants	Cocktail of humanized monoclonal antibodies possessing virus-neutralizing activity	Highly efficacious at a symptomatic (late) stage of infection. Should be stored at −20°C	[55, 57, 58]
Peptide-conjugated phosphorodiamidate morpholino oligomers (PMO)	Antisense oligonucleotides that block VP24 expression	High protective and therapeutic activity	[59, 60]
Antiviral siRNAs in a liposome form	*VP24* and *VP35* are the siRNA targets	High preventive activity (protection of healthcare workers and contacting persons)	[61, 62]
Peptide FX06 (GHRPLDKKREEAPSLR-PAPPPISGGGYR)	28-mer fibrin-derived peptide fragment (endothelial protector)	Therapeutic efficacy verified in one patient	[35, 49]

illustrated with the case of a WHO epidemiologist who worked for a long time in an EHF focus of Sierra Leone. When first signs of the disease appeared, the patient was treated according to a protocol accepted in malaria [25]. EHF was then diagnosed by a PCR test, and transfusions, ciprofloxacin, and metronidazole were administered [25]. On day 10 of illness, the patient was airlifted to Hamburg. He was admitted to an intensive care unit in a grave condition; therapy was aimed primarily at treating sepsis. The patient recovered and was discharged from the hospital on day 26. It should be noted that the patient did not receive antiviral drugs or the ZMapp cocktail. A conclusion was made that severe EHF cases can be treated successfully with conventional intensive care measures [25]. Thus, the availability of adequate intensive care and modern treatments for sepsis underlies successive therapy for EHF [25, 46].

However, it should be noted that the development of chemotherapeutics in modern pharmacology is focused to a great extent on commercial success and excess profits, as is evident from the fact that designing and testing antiviral drugs to inhibit *Ebolavirus* replication is of no interest to the majority of pharmaceutical companies worldwide and especially in Russia. It would be surprising indeed if Anaferon, Ingavirin, Kagotsel, and many other Russian drugs were proposed for treating highly dangerous infections, such as EHF. The main drugs used to prevent and treat EHF are summarized in Table 4.

Vaccines. It is clear that the primary task is to develop disease-preventing vaccines to ensure safety of healthcare workers and to perform large-scale vaccination in the endemic regions of West Africa for eradicating EHF. Attempts were also made to develop vaccines and vaccination protocols aimed at urgent disease prevention in contacting persons during the incubation period [51−56, 63]. More than ten vaccines are currently being developed and evaluated in clinical studies. Recombinant technologies are employed as a basis in constructing vaccines against the Ebola virus [52]. Recombinant vaccines are many and include those based on the vaccinia virus, Venezuelan equine encephalitis virus, type 3 parainfluenza virus, and several other common vectors.

The WHO supported vesicular stomatitis virus- and chimpanzee adenovirus (ChiAd3)-based vaccines expressing Ebola virus antigens [56, 64]. An Ad5-based vaccine was not considered to be promising because anti-adenovirus immunity is widespread in the population. A main drawback is that usual cold chain logistics is insufficient with all vaccines, which should be stored at extremely low temperatures (at liquid nitrogen temperatures in some cases). Such storage is problematic in African countries, rendering mass vaccination difficult and expensive.

Humanized antibodies. The ZMapp cocktail of humanized monoclonal antibodies neutralizes infection activity of the Ebola virus in the blood and tissues [57, 58] and occupies a special place among the bio-
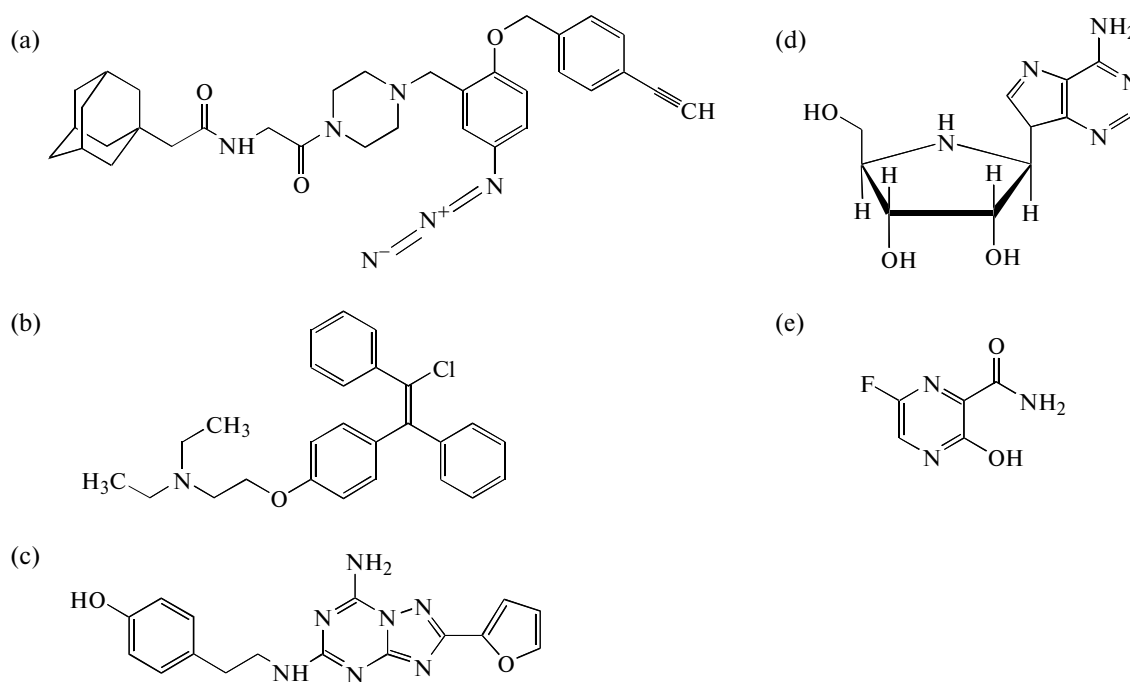
Fig. 5. Small molecules that are candidate drugs for EHF. (a) Niemann−Pick C1 cholesterol transporter blocker [65]. (d) Adenosine analog BCX-4430 [66]. (e) T-705 (pyrazine carboxamide), which inhibits viral RNA-dependent RNA polymerase [67−69]. (b) Estrogen receptor inhibitor clomiphene, which suppresses the endosomal stage of infection [70, 71]. (c) Adenosine A2b receptor antagonist ZM241385 [74].

logics listed in Table 4. It took at least 15 years of basic research to develop the drug. A technological implementation of the project is worthy of being among the best achievements of the 21st century [57] and is a source of pride for the Ministry of Health of Canada [58].

Pathogenesis-based drugs are aimed at controlling and managing the infection process to prevent dysfunction of vital organs and its sequels, including fatal outcomes. The drugs actually target the causes of fatal outcomes.

In their initial studies, researchers of the Vector State Research Center of Virology and Biotechnology were the first to try to design drugs that would correct the development of irreversible changes in affected tissues [32]. The unique drug Desferal was proposed to protect the lungs from hemorrhagic edema and consequent heme-induced oxidative stress [32]. Anti-TNFα antibodies were used for the first time to reduce the damaging effect of the cytokine storm [36].

A fibrin-derived peptide (a domain E1 fragment). Therapeutic use of peptide Bβ 15−42, which is known as FX06 and interacts with VE-cadherin, is of special interest. The peptide results from plasmin-catalyzed proteolysis of fibrin. Therapeutic efficacy of the peptide FX06 was studied in animals with myocardial reperfusion injury, which provides a model of myocardial infarction [35]. FX06 was shown to prevent an increase in vascular permeability in lipopolysaccharide-induced septic shock models. FX06 displayed good therapeutic properties in preventing the increased vascular perme-

ability syndrome in models with dengue virus-induced shock. Studies of the mechanism of action showed that the peptide FX06 prevents stress-induced activation of the RhoA-kinase [35]. It should be noted that FX06 has a high proline content (GHR*P*LDKKREEA*P*SLR*P*A-*PPP*ISGGGYR), suggesting a regulatory function towards PDZ-binding domains. Based on preclinical findings, FX06 was used to treat an EHF patient from Sierra Leone in Hamburg [25, 49]. Therapeutic success was attributed directly to the use of FX06 in this case [35, 49].

Apart from the above medicines, there are antiviral drugs based on small interfering RNAs or modified antisense oligonucleotides, combining certain advantages with considerable drawbacks [27, 28, 49, 55, 59−62, 64].

Recommendations for treating EHF patients with due regard to the new data on EHF pathogenesis and the causes of fatal outcomes were developed at the Institute of Influenza [46].

LOW-MOLECULAR-WEIGHT INHIBITORS OF EBOLA VIRUS REPLICATION

Antiviral therapy with broad-spectrum chemotherapeutics is a priority of special significance according to the WHO. Because high-level viremia accompanies EHF and the viral load correlates with the clinical course of the disease, etiotropic therapy aimed at suppressing virus replication is of utmost importance.

Great interest is attracted by the small molecules (low-molecular-weight compounds) that were tested in pre-clinical or Phase I clinical studies and showed efficacy in EHF. The set includes brincidofovir (CMX001), favipiravir (T-705), umifenovir, BCX-4430, FGI-103, triazavirine, ZM241385, and new triazolo purine and triazolo pyrimidine derivatives (Fig. 5). BCX-4430, FGI-103, T-705 [25, 28, 49, 64–71], and triazavirine [72, 73] are considered to be the most promising. Tri-azavirine is a triazolo triazine drug with broad-spectrum activity towards hemorrhagic fever viruses [73], although reference studies are necessary for testing its efficacy against the Ebola virus.

Adenosine receptor modulators and Toll-like receptor blockers were found to provide for a better survival of laboratory animals with sepsis accompanied by multiple organ failure [74]. New approaches help to substantially augment the set of drugs effective in EHF and other dangerous human diseases. A mass screening reveals substances with unexpected properties even among approved drugs [70, 71], and such drugs actually require only EHF to be approved as a new indication. A new mechanism of action was described for umifenovir (Arbidol), which consequently can be improved to serve as an Ebola virus replication inhibitor [75]. Studies at the Institute of Influenza made it possible to complete the design of antiviral agents of the series and to expand the activity spectrum of new compounds.

New information on the development of drugs and vaccines against the Ebola virus is available at many web sites, including those of the WHO, CDC, and Institute of Influenza.

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