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Ebola virus: unravelling pathogenesis to combat a deadly disease

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Ebola virus (EBOV) causes severe haemorrhagic fever leading to up to 90% lethality. Increasingly frequent outbreaks and the placement of EBOV in the category A list of potential biothreat agents have boosted interest in this virus. Furthermore, development of new technologies (e.g. reverse genetics systems) and extensive studies on Ebola haemorrhagic fever (EHF) in animal models have substantially expanded the knowledge on the pathogenic mechanisms that underlie this disease. Two major factors in EBOV pathogenesis are the impairment of the immune response and vascular dysfunction. Here, we attempt to summarize the current knowledge on EBOV pathogenesis focusing on these two factors and on recent progress in the development of vaccines and potential therapeutics.

Molecular biology of Ebola virus

Ebola viruses (EBOV) belong to the *Filoviridae* family (order *Mononegavirales*). The genus *Ebolavirus* (EBOV) is subdivided into four species: *Zaire ebolavirus* (ZEBOV), *Sudan ebolavirus* (SEBOV), *Ivory Coast ebolavirus* (ICEBOV) and *Reston ebolavirus* (REBOV) [1].

The 18.9-kb RNA genome of EBOV is non-infectious and encodes seven structural proteins and one non-structural protein in the following order within the genome: 3′ non-coding region (leader), nucleoprotein (NP), virion protein 35 (VP35), VP40, glycoprotein (sGP and GP) (see Glossary), VP30, VP24, RNA-dependant RNA-polymerase (L) protein and 5′ non-coding region (trailer). Mature EBOV particles form long filamentous rods with a uniform diameter of ~80 nm and a mean length of ~1250 nm (Figure 1a). Virus particles possess a central core, known as the ribonucleoprotein (RNP) complex, that consists of NP, VP35, VP30, L and the viral RNA (Figure 1b). This RNP complex is surrounded by a lipid envelope, with which the remaining proteins $GP_{1,2}$, VP40 and VP24 are associated; these three proteins function as surface glycoprotein, major matrix protein and minor matrix protein, respectively [2].

Epidemiology

Since its identification in 1976, there have been 1849 reported cases of Ebola haemorrhagic fever (EHF) including 1288 deaths (Table 1); all the outbreaks occurred in the tropical African ecosystem and were located between latitudes 5° North and 5° South. The epidemiology of human Ebola virus infections in nature is unknown. However, the time between the occurrence of index cases and the recognition of subsequent large outbreaks, in addition to the possible occurrence of asymptomatic infections, suggest that sporadic cases of unrecognized filovirus infections can go unnoticed [3] (Figure 1c).

Transmission of the disease generally results from contact with blood, secretions or tissues from patients or infected animals (e.g. gorillas and chimpanzees) [4]. It has been noted that many infections occurred as a result of injections with contaminated syringes, and that infections that are acquired in this way are normally fatal. Transmission of EBOV through mucosal exposure also occurs experimentally in non-human primates (NHPs) and, although it has never been shown in humans, it is believed that it is possible through contact between contaminated hands and the mucosa of the eyes [4]. Furthermore, there have been several cases in which transmission seems to have occurred from person to person by the airborne route [5]. However, this does not seem to be a major contributing mechanism for

Glossary

Adaptive immunity: components of the immune system that are acquired after birth. These are characterized by specific immune responses to an antigen, including antibody production by B cells, selection of active T cells, T-cell apoptosis and development of immunological memory.
Animal model: refers to an animal species that is sufficiently similar to humans in its response to an injury or disease so that it can be used in medical research to obtain information that can be extrapolated for human medicine.
Endothelial barrier: the interface that is formed by a layer of endothelial cells that lines the interior surface of blood vessels and controls the passage of materials in and out of the circulating blood supply.
Glycoprotein: a protein conjugated with one or more carbohydrate (sugar) components. The sugar residue(s) are typically attached to the protein at either asparagine residues (N-linked glycosylation) or at serine or threonine residues (O-linked glycosylation).
Innate immunity: all non-specific immune mechanisms by which pathogens are recognized and responded to. These rapid responses are coordinated through receptors that recognize a wide spectrum of conserved pathogenic components.
Interferon (IFN) antagonism: the process of evading the host response to IFN, including both immune activation and induction of an antiviral state. This can be achieved either by inhibition of IFN production or by blockade of IFN signalling in target cells and is known to be mediated by many viral proteins.
Pathophysiology: the disturbance of normal mechanical, physical and biochemical functions leading to or resulting from disease or injury.

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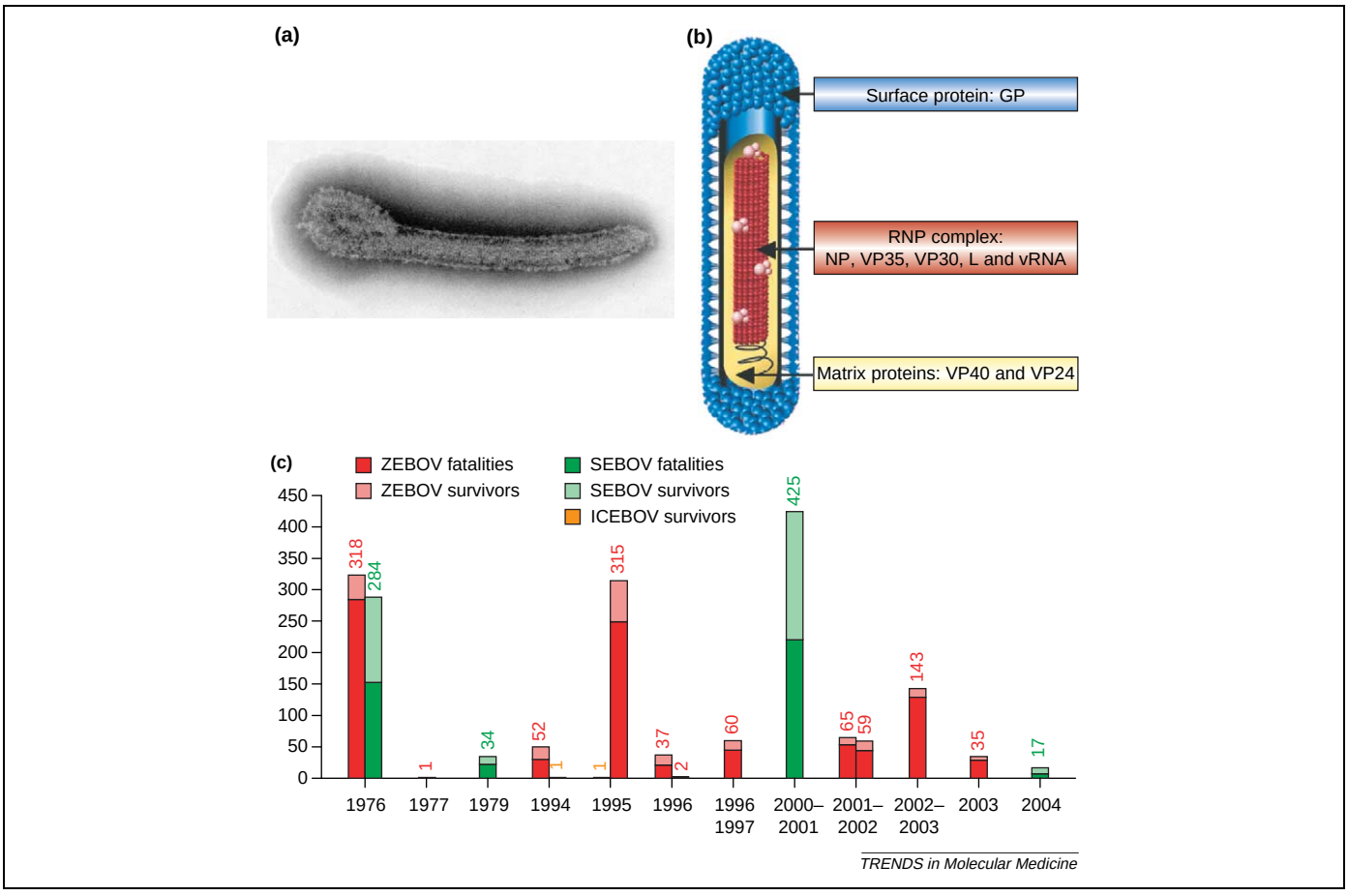

Figure 1. Structure and geographical distribution of Ebola virus. **(a)** Electron micrograph of an EBOV particle (kindly provided by L. Kolesnikova). **(b)** Structure of an EBOV particle (kindly provided by S. Bamberg). The RNP complex is made up of the viral proteins NP, VP35, VP30, L and the viral RNA (red); the matrix space is made up of the proteins VP40 and VP24 (yellow); the viral envelope and the surface protein GP are shown in blue. **(c)** Overview of cases and fatality rate of EBOV outbreaks. The number of cases in each outbreak is indicated above the bars.

transmission because all epidemics to date have been successfully controlled using isolation techniques without specific airborne precautions.

The source of EBOV has remained elusive since their initial discovery. However, a recent survey on small vertebrates that were collected during EBOV outbreaks in 2001 and 2003 in Gabon and the Republic of Congo has shown evidence of apparent asymptomatic infection in three species of fruit bats [6]. This supports earlier experimental data that demonstrated replication of EBOV in bats [7]. Further laboratory and ecological investigations will be required to determine the relevance of this finding.

Clinical presentation

EBOV infection in humans and NHPs results in a particularly virulent form of viral haemorrhagic fever. Following an incubation of 4–10 days [2], fever of $>38.3°C$ abruptly develops. Additional early symptoms are non-specific and can include chills, muscle pain, nausea, vomiting, abdominal pain and/or diarrhoea [8–10]. Swelling of the lymph nodes, kidneys or brain, as well as necrosis of the liver, lymph organs, kidneys, testis and ovaries can occur. All patients show some extent of impaired coagulation, which can manifest as conjunctival haemorrhage, bruising, impaired clotting at venipuncture sites and/or presence of blood in the urine or faeces. In fatal cases, gross pathological changes include visceral organ necrosis and haemorrhage into the skin, mucous membranes, visceral organs or the lumen of the stomach and/or intestines [10]. Although ~50% of individuals develop a maculopapular rash on the trunk and shoulders, profuse bleeding is rare and, when it occurs, it is mainly restricted to the gastrointestinal tract [11]. Severe nausea, vomiting and prostration, in addition to increased respiration rate, anuria and decreased body temperature all indicate impending shock and suggest a poor prognosis [8]. Case-fatality rates that are associated with EHF infection range between 50% and 90% and mainly depend on the virus species, with ZEBOV being the most virulent [2,12]. In addition, the virus load between fatal and non-fatal cases differs by ~2 $\log_{10}$. Virus loads in peripheral-blood samples reach peak titres of 3.4×10^9 genome copies per ml of blood in fatal cases and 4.3×10^7 genome copies per ml of blood in non-fatal cases [13,14]. In fatal cases, high virus loads (i.e. $>1\times10^8$ genome copies per ml of blood) are reached as early as two days after the onset of the disease [13], and death occurs 6–16 days after the onset of symptoms [2]. In addition, among survivors a protracted period of convalescence is common and presents various sequelae including deafness, athralgia, pericarditis and orchitis [8]. EBOV can persist in

Table 1. Ebola outbreaks[a]

Year	Species	Country	Cases	Fatality	Situation
1976	Zaire	DRC[b]	318	88%	Yambuku area
1976	Sudan	Sudan	284	53%	Nzara and Maridi areas
1977	Zaire	DRC[b]	1	100%	Tandala village (retrospective)
1979	Sudan	Sudan	34	65%	Nzara area
1989	Reston	USA	0	0%	Macaques were imported into Virginia, Texas and Pennsylvania. Four humans developed antibodies but no illnesses were reported
1990	Reston	USA	0	0%	Macaques were imported into Virginia and Texas. Four humans developed antibodies but no illnesses were reported
1992	Reston	Italy	0	0%	Epizootic in macaques imported into Siena
1994	Zaire	Gabon	52	60%	Mékouka and other gold-mining camps. Originally identified as yellow fever
1994	Ivory Coast	Ivory Coast	1	0%	Scientist who performed autopsy on a dead wild chimpanzee; treated in Switzerland
1995	Ivory Coast	Liberia	1	0%	Infection in a refugee from the Liberian civil war; identified based on serology only
1995	Zaire	DRC[b]	315	79%	Kikwit area. Index case who worked in forest adjoining city
1996	Zaire	Gabon	37	57%	Mayibout area. Early cases were involved in the butchering of a dead chimpanzee
1996-1997	Zaire	Gabon	60	75%	Booué area. Index case was a hunter living in a forest camp
1996	Zaire	South Africa	2	50%	Medical professional travelling from Gabon after treating infected individuals. The nurse who treated him died
1996	Reston	USA	0	0%	Macaques were imported into Texas
1996	Reston	Phillipines	0	0%	Identified in a monkey export facility
2000-2001	Sudan	Uganda	425	53%	Gulu, Masindi and Mbarara districts. Major risk factor was funeral attendance
2001-2002	Zaire	Gabon	65	82%	Ogooue–Invindo province
2001-2002	Zaire	RC[c]	59	75%	Mékambo, Mbomo and Kéllé districts
2002-2003	Zaire	RC[c]	143	90%	Mbomo and Kéllé districts
2003	Zaire	RC[c]	35	83%	Mbandza and Mbomo districts
2004	Sudan	Sudan	17	41%	Case recognition was complicated by co-circulation of haemorrhagic measles

[a]From WHO Media Centre Fact Sheet Ebola Haemorrhagic Fever (www.who.int/mediacentre/factsheets/fs103/en/).
[b]Democratic Republic of Congo.
[c]Republic of Congo.

immunologically privileged sites, and has been isolated from seminal fluid for $\leq$82 days after the onset of symptoms and detected by reverse-transcription polymerase chain reaction (RT-PCR) for $\leq$101 days after the onset of symptoms [15].

Pathogenesis

Animal models

In recent years, there has been significant progress towards the understanding of the pathogenic mechanisms that underlie EHF. However, there are only limited data regarding the pathophysiology of EHF in humans owing to the occurrence of outbreaks in remote areas and the lack of facilities that enable safe and thorough investigations during an outbreak [16,17]. Therefore, the development of animal models for EHF has been invaluable in increasing the knowledge of EBOV pathogenesis. At present, EHF can be best reproduced in NHPs, but also in mice and guinea pigs (Table 2). Notably, wild-type EBOV (EBOV-WT) is not lethal in these rodent models but can become fatal through passaging of the virus [18–20]. In both mouse and guinea pig models, increased virulence has been associated with mutations in NP and VP24 [21,22]. Although these animal models have significantly helped enhance the knowledge on EBOV pathogenesis, some aspects of the human form of the disease are not authentically replicated. One has to keep in mind that there are considerable differences between rodent and human immunology [23]. Neither mice nor guinea pig models show marked haemorrhagic manifestations, which is a hallmark of EBOV infection in humans. Also, bystander apoptosis of lymphocytes, which is observed in humans, is not prominent in rodents [19,20,24]. NHPs model these aspects of the human disease; however, the typical time of death in the NHP model is 6–9 days after exposure to ZEBOV infection, whereas in the human infection the time of death is prolonged, averaging between 6–16 days after onset of symptoms [25–27]. The shorter course of the disease in NHPs is not accompanied by the development of antigen-specific immunity, whereas in human cases an adaptive immune response might contribute to survival [28]. However, experimentally infected NHPs are normally exposed to higher doses of EBOV than humans would be during a natural exposure, which makes the comparison difficult.

Besides these animal models, *in vitro* studies have also contributed to expand the knowledge of EBOV pathogenesis. In this respect, very important was the development of a reverse genetics system for EBOV, which enables the creation of infectious viruses from cloned cDNA and, thus, the generation of virus mutants [29,30]. The application of this technology to viruses is reviewed elsewhere [31].

Table 2. Animal models of Ebola virus infection compared with the human disease

	Mouse	Guinea pig	NHP	Human[a]
Adaptation required	Yes	Yes	No	No
Macular rash	No	No	Yes	Yes
Haemorrhagic manifestations	Not profound	No	Yes	Yes
Coagulation abnormalities	Not profound	Conflicting data	Yes	Yes
Liver enzymes	Elevated	Elevated	Elevated	Elevated
Thrombocytopenia	Yes	Yes	Yes	Yes
Bystander apoptosis	No	No	Yes	Yes
Cytokine response	Yes	Yes	Yes	Yes
Time to death	4–6 days[b]	6–9 days[b]	6–9 days[b]	6–16 days[c]
Required infrastructure and/or costs	Moderate	Moderate	Very high	NA[d]
Ethical concerns	Moderate	Moderate	High	NA[d]

[a]Information about the human disease is provided for comparison; it has to be kept in mind that it is problematic to compare naturally occurring disease with an experimental model.
[b]Time of death after infection.
[c]Time of death after onset of symptoms.
[d]NA, not applicable.

Target cells and cell entry

The current model for the course of EBOV infection (Figure 2) is that the virus enters the host through minute lesions in the skin and the mucosa and infects its primary target cells – macrophages and dendritic cells (DCs) [32]. It has been shown that human macrophages can be infected *in vitro* by EBOV [33], and that EBOV can be found in macrophages from deceased human patients [34]. In addition, serial-sacrifice studies in NHPs have shown that macrophages and DCs are early targets during the infection; indeed, infected macrophages are found in these animals as early as two days post infection (p.i.) [27,35]. Macrophages are primary target cells in mice [19,36] and guinea pigs [18,20], and are the only cells that were shown to be infected by EBOV-WT in the latter model [18].

Also endothelial cells can support EBOV replication *in vitro* [37,38]; infected endothelial cells have been found in deceased human patients, NHPs and mice as well as in guinea pigs that were infected with guinea-pig-adapted virus [18–20,27,36,38,39]. However, it is generally believed that these cells are infected late during the course of the disease and are less affected than macrophages or DCs.

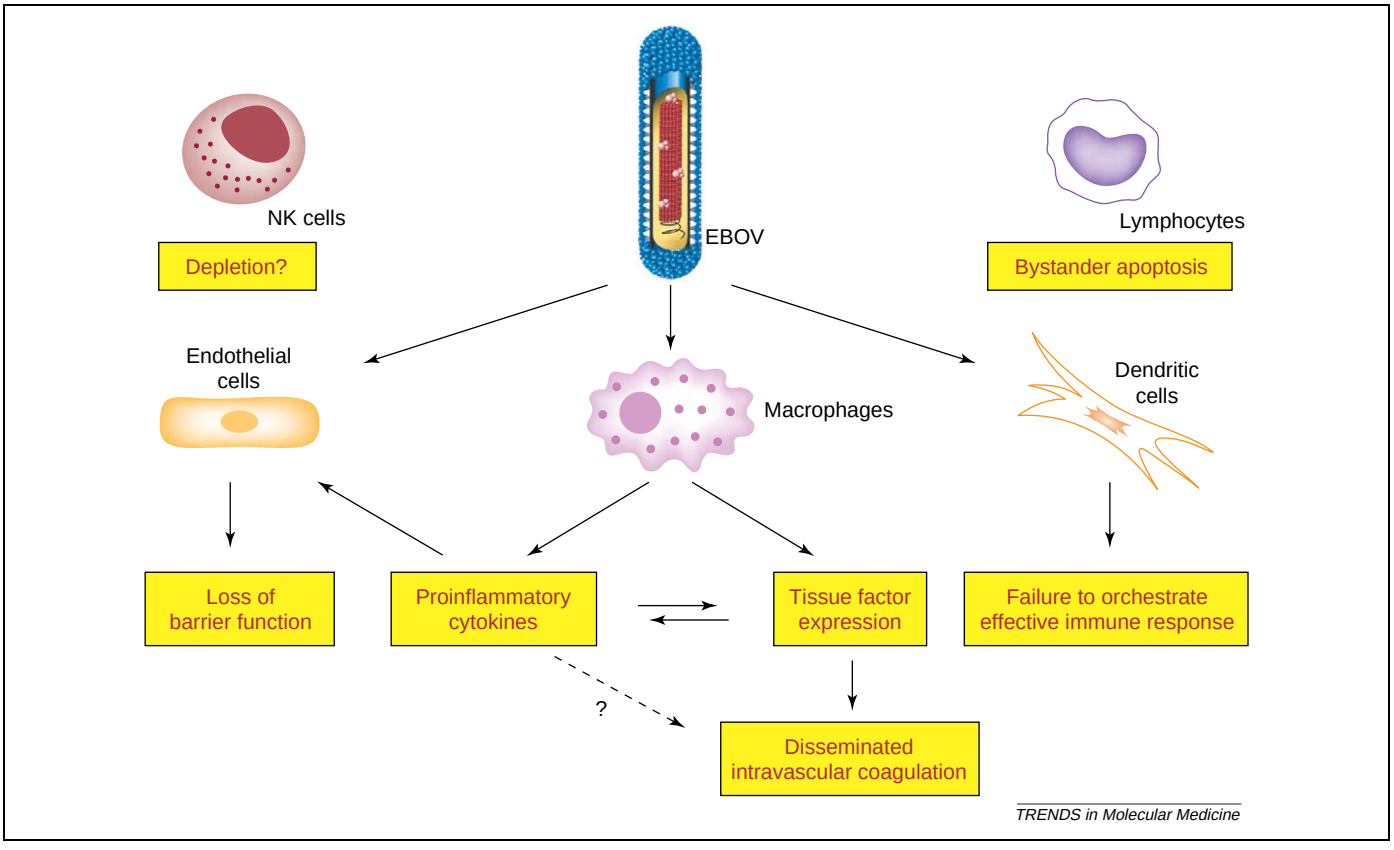

Figure 2. Overview of the mechanisms that are involved in EBOV pathogenesis. Primary target cells for EBOV are macrophages and dendritic cells (DCs). Infection of DCs inhibits their function. Macrophages become activated and produce proinflammatory cytokines and tissue factor (TF). Endothelial cells are infected by EBOV and activated by the produced cytokines; these events lead to a loss of their barrier function. The expression of TF and probably also of the produced cytokines contribute to disseminated intravascular coagulation. Lymphocytes and natural killer (NK) cells are not infected by EBOV but undergo bystander apoptosis or are depleted by an as yet unclear mechanism.

Several proteins might act as cellular attachment molecules for EBOV. The C-type lectin asiaglycoprotein receptor mediates infection of hepatocytes by the closely related Marburg virus, and enhances infection by pseudotypes bearing GP of EBOV *in vitro* [40,41]. Other proteins that might act as cellular attachment molecules are: (i) the β1-integrin receptor; (ii) the C-type lectins DC-specific (DC) or liver and/or lymph-node-specific (L) intercellular adhesion molecule 3 (ICAM3) grabbing nonintegrin (DC-SIGN and L-SIGN); (iii) the liver and lymph node sinusoidal endothelial cell C-type lectin (LSECTin); (iv) the human macrophage galactose-specific and N-acetylgalactosamine-specific C-type lectin (hMGL); and (v) DC-SIGN-related factors (DC-SIGNR) [17]. Based upon these findings, it has been recently suggested that EBOV uses not one entry mechanism but various C-type lectins for efficient attachment to host-cell types, which would explain its broad cell tropism [17,42].

Impairment of innate immunity

A central role for the innate immune system in EBOV infection has been demonstrated by many studies (Figure 2). In humans and in NHPs, inflammatory responses that are accompanied by substantial cytokine production can be detected as a result of EBOV infection [17,43]. Also, the interferon (IFN) response is very important for the outcome of the disease in mice [44]. Although adult immunocompetent mice are resistant to EBOV-WT, they die within a week if they are infected with EBOV-WT and treated with anti-IFN antibodies. Also, mice that lack either the IFN-α/β receptor or the signal transducer and activator of transcription-1 (STAT-1), which is involved in IFN signalling, are susceptible to EBOV-WT [44]. In contrast, severe combined immunodeficient (SCID) mice that lack both humoural and cellular adaptive immune responses succumb very slowly to EBOV-WT infection and show a clinical picture that does not resemble the infection in humans or NHPs [44]. *In vitro*, EBOV selectively suppresses responses to IFN-α and IFN-γ and the production of IFN-α in response to double-stranded RNA [37,45,46]. Basler *et al.* [47,48] identified two EBOV proteins that interfere with the IFN response. VP35 blocks phosphorylation of the IFN-regulatory factor 3 (IRF3), which acts as a transcription factor for IFN production, whereas VP24 seems to block IFN signalling. Recently, Halfmann *et al.* [49] indicated that the block of IFN signalling might be due to inhibition of p38 phosphorylation, which is central in the mitogen-activated-protein-kinase (MAPK) p38 IFN-signalling pathway. The role of IFN antagonism *in vivo* has not yet been extensively analysed. In one study in NHPs, treatment with high doses of IFN had only minor benefits, thus suggesting that blockade of IFN signalling has an important role in the pathogenesis of EBOV infection *in vivo* [50]. However, in this study only one isoform of IFN was administered, which might be insufficient to elicit an antiviral response.

As previously mentioned, monocytes, macrophages and DCs are the most important early target cells during EBOV infection (Figure 2); however, to what extent infection of monocytes and macrophages impairs function of these cells has not been extensively studied. In contrast, DCs, which have a crucial role in both innate and adaptive immunity, fail to fulfill their function after infection with EBOV *in vitro*. Infected DCs do not produce proinflammatory cytokines or express costimulatory molecules such as CD80 or CD86, are impaired in their ability to support T-cell proliferation and undergo anomalous maturation [51,52]. Because non-infectious EBOV virus-like particles (VLPs) can elicit these responses, the infectious virus is likely to interfere actively with the function of DCs [53]. Similarly, DCs show no increase in the expression of CD80 or CD86 following infection in a NHP model, confirming the *in vitro* data [54]. Furthermore, EBOV can induce the pro-apoptotic tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) in DCs both *in vitro* and *in vivo* [35,55]. However, there is no evidence of apoptosis in EBOV-infected DCs *in vivo* [35].

Another group of innate immune cells that are affected by EBOV infection are the natural killer (NK) cells (Figure 2). These cells respond in an antigen-independent manner to viral infections and kill infected cells by releasing perforin and granzymes, and by inducing apoptosis [56]. Although NK cells do not seem to be infected by EBOV, their number dramatically drops during the course of an EBOV infection in NHPs and they almost completely disappear by day 4 p.i. [24,54]. High levels of caspase activity in NK cells, DNA fragmentation in lymphocytes, the release of 41/7 nuclear-matrix protein into the plasma and evidence from electron microscopy all suggest that the decline in NK-cell number is due to apoptosis [24,54,57]. In humans, there is evidence of extensive intravascular apoptosis together with disappearance of CD3, CD8 and T-cell receptor (TCR)-Vβ mRNA from peripheral blood mononuclear cells (PBMCs). However, based on these data it is not possible to determine whether the depleted cells are $CD8^+$ NK cells or $CD8^+$ cytotoxic T cells [57]. In another study, it was shown by flow cytometry that the number of NK cells is only slightly reduced in infected patients [14].

Function and impairment of adaptive immunity

The role of adaptive immunity in EBOV infection is harder to assess than that of innate immunity because of the difficulty to obtain relevant data from the current animal models. It has been noted earlier that NHPs succumb to the disease 6–9 days p.i., whereas in humans the longer incubation period and the longer course of disease enable an adaptive immune response to be mounted [28]. To date, the limited data that are available from human infections show profound differences in the adaptive immune responses of fatal and non-fatal cases, thus indicating an important role of the adaptive immune system during EBOV infection [14,57]. Survivors show specific immunoglobulin (Ig)M antibodies as early as two days after the onset of symptoms and IgG antibodies 5–8 days after the onset of symptoms. In contrast, in fatal cases low levels of specific IgM are detected in only 30% of patients, whereas specific IgGs are never detected [57,58]. Also, differences seem to exist in the pattern of T-cell activation between fatal and non-fatal cases [14,57]. Although data differ

between studies, one common observation is the decrease of T-cell numbers in fatal cases prior to death [14,57].

In accordance with the data that have been obtained in human patients, a strong depletion of both $CD4^+$ and $CD8^+$ lymphocytes and plasma cells can be found in NHPs [24,54]. Interestingly, similar to NK cells, lymphocytes are not infected by EBOV but undergo 'bystander apoptosis' [24] (Figure 2).

Because differences in the adaptive immune response occur early after the onset of symptoms, it has been suggested that factors early in infection determine the outcome [57]. This hypothesis is supported by the finding that an early inflammatory response also correlates with survival [43]. Further studies that analyse the influence of EBOV on both innate and adaptive immunity, and especially on the interaction between the two, will be necessary to understand the role of immunosuppression during EBOV infection. Of particular interest will be the role that impairment of DCs, which are crucial for these interactions, has in the pathogenic process.

Vascular dysfunction

Vascular dysfunction and loss of endothelial barrier function are considered to be major contributors to the fatal outcome of EBOV infections [59]. It has been suggested that vascular dysfunction is caused by activation rather than direct infection of the endothelium [60]. Treatment with tumour necrosis factor-α (TNF-α), elevated levels of which are associated with fatal EBOV infection [61], or with the supernatants of monocyte/macrophage cultures that are infected with the closely related Marburg virus increases permeability of cultured human endothelial cell monolayers [61–63]. Also, VLP-associated GP can activate endothelial cells and cause a breakdown of their barrier function, further supporting this hypothesis [63] (Figure 2).

Another proinflammatory mediator that is likely to contribute to endothelial dysfunction is nitric oxide (NO), which is a potent endogenous vasodilatator and is involved in the development of vasodilatory shock [64]. In EBOV-infected NHPs, elevated nitrate levels that indicate increased production of NO can be detected already at day 3 p.i. [35,55]. Also, highly increased levels of NO are found in human blood and they correlate with the fatal outcome of EHF [14,65].

Besides its impact on endothelial-barrier function, TNF-α can induce the expression of tissue factor (TF) in endothelial cells [66] (Figure 2), and can impair the function of the anticoagulant-protein-C pathway by down-regulating thrombomodulin [67]. In EBOV-infected NHPs, plasma levels of protein C dramatically drop shortly after infection (day 2 p.i.) and increased TF mRNA levels can be detected already at day 3 p.i. [68]. TF is present on infected macrophages, endothelial cells and on the surface of membrane microparticles that are present in copious amounts in the blood of infected NHPs. *In vitro*, EBOV can directly induce the expression of TF on the surface of macrophages [68] (Figure 2). Increased levels of TF, whether induced by TNF-α or by direct infection of macrophages, might lead to the development of disseminated intravascular coagulation (DIC), which is a prominent feature of EHF. DIC is characterized by the systemic activation of the blood-coagulation system, thereby leading to fibrin deposition and microvascular thrombi [67]. Inhibition of TF increases the survival time in EBOV-infected NHPs, which further indicates the significance of TF in the pathogenesis of EBOV infection [69].

The pro-coagulant state that is induced by filovirus infection not only directly harms the host by causing DIC but also enhances inflammation through several mechanisms, which, in turn, further provoke activation of coagulation [17,67]. This two-way interaction between coagulation and inflammation is demonstrated by the fact that EBOV-infected NHPs that are treated with an inhibitor of TF show decreased levels of interleukin-6 (IL-6) and macrophage chemotactic protein-1 (MCP-1) [69].

The role of viral glycoproteins in the pathogenesis of EHF

It has been suggested that viral glycoproteins have an important role in the pathogenesis of EHF [70] (Figure 3). It has been hypothesized that EBOV transmembrane glycoprotein $GP_{1,2}$ is the main viral determinant of vascular cell cytotoxicity and injury, and that haemorrhage is a consequence of replication and $GP_{1,2}$-expression-induced damage of endothelial cells [71]. Indeed, *in vitro* expression of $GP_{1,2}$ caused detachment of cells, and expression of $GP_{1,2}$ in vessel explants led to increased permeability [71]. However, it has been later shown that the detachment of cells was not due to cell death [72,73]. As previously mentioned, infection of endothelial cells is a late event in the course of infection; replication of EBOV in endothelial cells of NHPs can first be detected at day 5 p.i., whereas DIC and perivascular oedema are found at day 4 p.i. along with ultrastructural changes in endothelial cells – namely non-viral tubuloreticular inclusions [38]. Thus, a major role of virus infection of endothelial cells and/or a direct cytopathic effect mediated by $GP_{1,2}$ in the development of haemorrhage is questionable.

In addition to the structural $GP_{1,2}$, EBOV expresses four soluble glycoproteins: (i) the soluble glycoprotein sGP; (ii) Δ-peptide, the smaller cleavage fragment of the precursor of sGP; (iii) GP1, the larger cleavage fragment of the transmembrane glycoprotein $GP_{1,2}$; and (iv) $GP_{1,2\Delta}$, which is the product of a metalloprotease cleavage of surface expressed $GP_{1,2}$ [70,74–76], all of which might take part in the impairment of the immune system. It was proposed that sGP binds to neutrophils through their $F_C\gamma$ receptor III and, consequently, inhibits their activation [77,78]; however, this concept has been challenged by several reports [79,80]. It was further suggested that both sGP and $GP_{1,2\Delta}$ act as decoys for antibodies [74,81] although the role of sGP as a decoy is controversial, because it has a different quaternary structure than $GP_{1,2}$ [74]. Nonetheless, the neutralizing effect of antiserum against $GP_{1,2}$ and sGP is markedly reduced in the presence of sGP [81]. Recently, another novel role for sGP in the pathogenesis of EHF has been proposed [63]: $GP_{1,2}$ containing VLPs, but not recombinant sGP or the Δ-peptide, can impair the barrier function of endothelial cells *in vitro*, an effect that is further increased by the

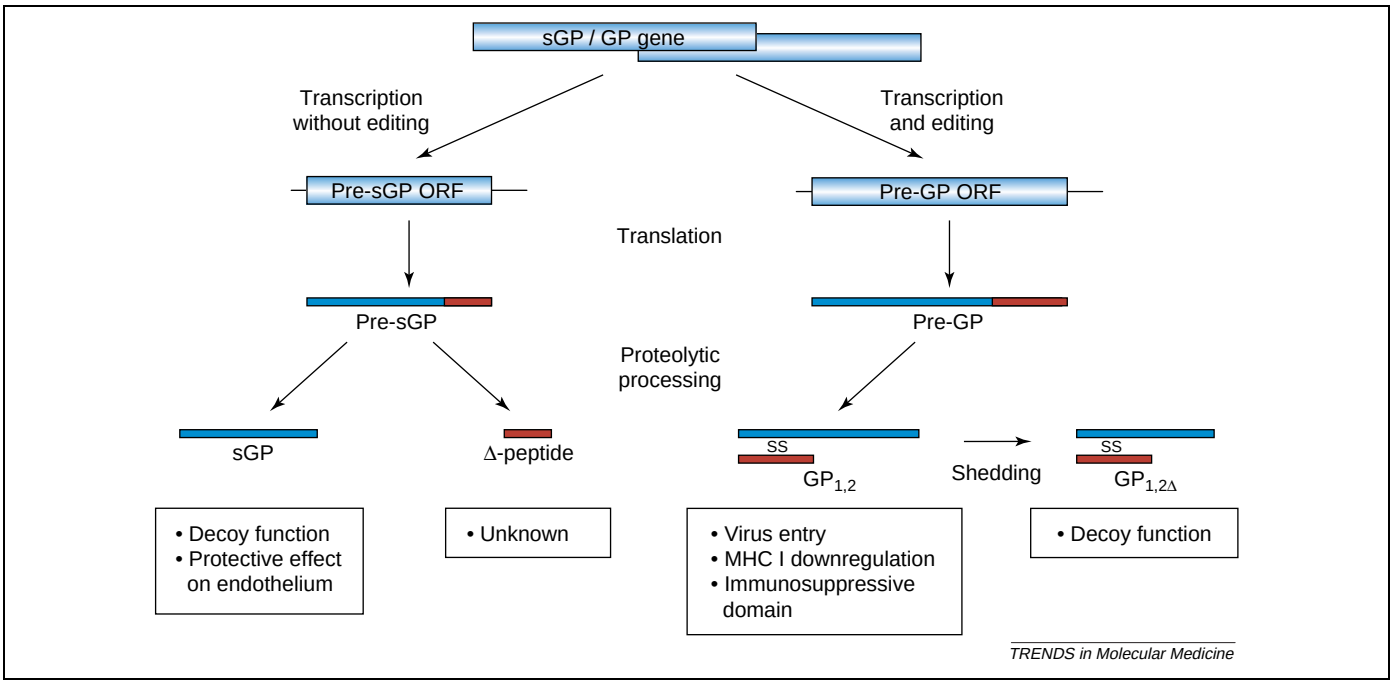

Figure 3. Expression strategy for EBOV glycoproteins and their proposed role in pathogenesis. sGP and Δ-peptide are produced by cleavage of a precursor glycoprotein that is encoded by the open reading frame (ORF) on gene 4. Pre-GP is also encoded by gene 4 after an insertion of an adenosine residue in the mRNA during transcription (RNA editing). Subsequently, pre-GP is proteolytically processed into $GP_{1,2}$, which can be further processed by an extracellular metalloproteinase into the soluble $GP_{1,2\Delta}$. Proposed roles for the different forms of GP in pathogenesis are indicated.

presence of TNF-α. sGP can reverse the effect of TNF-α treatment and partially restore endothelial barrier function [63]. Further studies will be necessary to elucidate the significance of a protective role of sGP *in vivo*.

Another mechanism that might contribute to the pathogenesis of EHF is the presence of a putative immunosuppressive domain close to the C-terminus of $GP_{1,2}$ [82]; peptides that simulate its structure inhibit blastogenic-lymphocyte proliferation and NK-cell activity [83]. However, the role for this C-terminal motif in the context of the glycoprotein remains to be determined. Finally, EBOV GP might contribute to immunosuppression by downregulating expression of the major histocompatibility complex class I (MHC-1) on the surface of cells, as has been observed *in vitro* [46,72].

Treatment and vaccines

Treatment

At present, the treatment for EHF is mainly supportive and involves a combination of intravenous-fluid replacement, administration of analgesics and standard nursing measures [25].

Despite the lack of any specific antiviral drugs for the treatment of EHF, a few experimental approaches have shown promise in recent years. In particular, because overexpression of TF has such a profound effect in the development of DIC, the possibility of inhibiting this pathway has been considered as a therapeutic approach. Despite reservation towards the use of anticoagulants in the treatment of haemorrhagic fever disease [84], Geisbert *et al.* [69] showed that the recombinant nematode anticoagulant protein c2 (rNAPc2), administered as late as 24 hours p.i., produced a 33% survival rate in an otherwise uniformly lethal EBOV-infected NHP model. Moreover, the survival time in the remaining animals was significantly prolonged indicating that, although this therapy might not be sufficient on its own, it might be a valuable tool in the treatment of EHF and other haemorrhagic diseases that involve overexpression of pro-coagulant molecules.

Another possible treatment that has been successfully applied to viral haemorrhagic fevers is the use of passive immunization [84]. However, its usefulness in the treatment of EHF remains unclear. Several reports have indicated that this approach might be valuable in rodent models of EHF [25], although transfer of serum from animals that were vaccinated with EBOV NP and GP-containing Venezuelan equine encephalitis (VEE) replicon was not protective in guinea pigs [85]. Results of passive-transfer experiments that used a horse hyper-immune serum in NHPs also produced inconsistent findings. In hamadryl baboons (*Papio hamadryas*), treatment was protective against $\leq$110 LD_{50} of EBOV challenge [86]. However, in cynomolgus macaques (*Macaca fascicularis*) treatment only delayed death but did not prevent it [87]. During the outbreak in Kikwit in 1995 (Democratic Republic of Congo), sera from convalescent EBOV patients were transfused into eight severely ill patients that exhibited haemorrhagic manifestations as a result of infection [88]. Of these patients, all but one survived; however, it is unclear to what extent the selection of these patients from the late stage in the outbreak and/or the intensive supportive care they received contributed to their survival [88].

Vaccines

Early attempts to produce vaccines for EBOV focused on the use of the inactivated virus and were universally

unsuccessful [4,26]. More recently, several vaccine strategies using recombinant viruses and/or DNA vaccination have been developed. Although these strategies were successful in protecting rodents from EBOV, almost none of them was successful in protecting NHPs [26]. VLPs have also been successfully used to vaccinate mice and guinea pigs, but have not yet been tested in NHPs [89]. The first vaccine of proven efficacy in NHPs was a combined DNA-prime and adenovirus-boost approach; however, this protocol required >6 months to provide protective immunity and is, therefore, of limited utility [90]. However, subsequent studies using only a single dose of NP and GP-expressing recombinant adenovirus protected NHPs against a high challenge dose (1500 LD_{50}) of EBOV just 28 days after immunization, indicating that this strategy could be useful in the context of an outbreak [91]. However, a problem that will need to be addressed is that pre-existing immunity can severely compromise the efficacy of a vaccine that is based on human adenoviruses [92].

Another candidate system that is based on a recombinant vesicular stomatitis virus (VSV) has been successful in both rodent and NHP models of EBOV infection. These viruses were produced by replacing the VSV-GP open reading frame (ORF) with the full-length GP of EBOV in the VSV infectious clone system [93]. The recombinant viruses demonstrate the tropism of EBOV, but are highly attenuated *in vivo*. To date, a single vaccination with the VSV–EBOV recombinant vaccine has been shown to protect NHPs 28 days after vaccination [94]. Because the VSV vaccine platform is based on a recombinant virus, there are some concerns about its safety for use in humans, which might make it difficult to get approval for such a vaccine from regulatory bodies.

More recently, another recombinant vaccine that is based on human parainfluenza virus has been shown to protect guinea pigs from EBOV infection after a single intranasal inoculation [95]. This approach seems promising but might have limitations similar to the adenovirus approach due to pre-existing antibodies in humans.

Concluding remarks

EBOV is a highly pathogenic virus that has caused an increasing number of outbreaks in central Africa in the past decade. Because of its high fatality rate and potential use as a bioweapon, it is very important to understand its mechanisms of pathogenesis and, ultimately, to develop vaccines and therapeutics.

The current model for EBOV pathogenesis is that, after entering the host, EBOV targets macrophages and DCs, thereby inducing an inflammatory state with high levels of proinflammatory cytokines (Figure 2). At the same time, the virus evades the immune response by several mechanisms including IFN antagonism, depletion of NK cells and lymphocytes and impairment of DC function. The produced cytokines and the direct infection of macrophages trigger the expression of TF, thus provoking a pro-coagulant state, which in turn further enhances inflammation. The pro-coagulant state develops into DIC and the released cytokines impair endothelial barrier function, which together might lead to severe shock and death.

Box 1. Outstanding questions

- What are the host factors that contribute to Ebola virus pathogenesis?
- What are the virus-encoded virulence factors?
- What is the molecular basis for the difference in virulence that is observed among Ebola virus species?
- What are the mechanisms that underlie vascular instability and coagulopathy during Ebola virus infection?
- What are the mechanisms that cause immunosuppression, and how does immunosuppression contribute to pathogenesis?
- Can an understanding of Ebola virus pathogenic mechanisms help to develop successful therapeutic and/or prophylactic approaches to control Ebola virus infection?

Although there are currently no approved vaccines or treatments for EHF, rNAPc2 and at least two recombinant virus vaccine approaches have shown promise in the NHP model. Further research into the molecular details of the viral life cycle and virus–host interactions will help develop further approaches to counter EHF (Box 1). Of special interest will be to understand the pathogenic mechanisms that underlie vascular instability, coagulopathy and immunosupression (e.g. depletion of lymphocytes and impairment of DCs). Another interesting aspect is to understand the molecular basis for the difference in virulence that is observed among different EBOV species (e.g. ZEBOV and REBOV), with REBOV being apparently non-pathogenic in humans. Finding the molecular determinants for this difference will help identify virulence factors and potential targets for therapeutic interventions.

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