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Descriptive Analysis of Ebola virus Proteins: Towards Development of Effective Therapeutics and Vaccines

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Authors' contributions

This work was carried out in collaboration between both authors. Author SMM wrote the first draft. Both authors SMM and GK managed the literature searches, read and approved the final manuscript.

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Review Article

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ABSTRACT

Ebola virus (EBOV) is a non-segmented enveloped RNA virus. It has seven structural proteins and three non-structural proteins (Δ-peptide, ssGP and sGP). Structural proteins include viral proteins (VP24, VP30, VP35, and VP40), glycoprotein(GP), nucleoprotein(NP) and RNA polymerase L. The functions of structural proteins range from replication, transcription and maintenance of the structural stability of EBOV. VP24 and VP35 are well known for suppressing host interferon (IFN) response systems. There are no approved treatments for Ebola virus disease (EVD). However, several virus vectored vaccines aimed at inducing B and T cell immunity against GP and NP are currently under development or undergoing clinical trials. Unlike virus vectored vaccines, virus like particle (VLP) based vaccines have been developed to elicit immunity against GP, NP, and VP40. They can also be engineered to include VP24 and VP35. Several compounds such as small interfering RNAs (siRNAs), phosphorodiamidate morpholino oligomers (PMOs), c3-NpcA, peptides, NSC629, E64, compound 3.47, compound 7, CA074, and Ca-c3 Ado that target different stages of the EBOV replication cycle have been found to be effective. Other compounds such as recombinant

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nematode anticoagulant protein c2 (rNAPc2) and Recombinant human activated protein C (rhAPC) have been designed to reduce coagulopathies seen in late stages of EVD. Cocktails of neutralising monoclonal antibodies are also effective against EBOV alone or in combination with IFNs. Therefore, this review discusses the structure and mechanism of action of EBOV proteins, current promising therapeutics and vaccines against EBOV, and different ways of improving their efficacy.

Keywords: Non-structural proteins; VP40; VP35; VP24; VP30; GP; L protein; therapeutics; vaccines.

1. INTRODUCTION

The current outbreak of Ebola virus disease (EVD) in West Africa has led to a surge in public interest and concern regarding Ebola virus (EBOV). The recent situation report from the world health organisation (WHO) covering the period March, 2014 to 4March, 2015 shows that the disease has claimed 9,807 lives and infected 23,969 people [1]. EBOV is an enveloped, negative sense, single stranded RNA virus. It has seven structural proteinsand three nonstructural proteins [soluble GP (sGP), secreted soluble GP (ssGP) and ∆-peptide [2]. The functions of nonstructural proteins are unknown. Structural proteins include glycoprotein (GP), nucleoprotein (NP), RNA polymerase L, and viral proteins (VP24, VP30, VP35, and VP40) [2]. GP is responsible for binding to host cell surface receptors, membrane fusion and viral entry [2]. NP and VP30 along with VP35 and RNA polymerase L interact with EBOV RNA genome to form the ribonucleocapsid complex [2-3]. All the ribonucleocapsid complex-associated proteins are involved in transcription and replication of the genome, whereas the envelope-associated proteins (GP, VP24 and VP40) play a role in virion assembly, budding and virus entry [2-3]. The functions of VP24, VP35 and to a lesser extent VP40 as suppressors of host interferon (IFN) signalling pathways are well characterised [2].

It is believed though with little available evidence that humans initially contract the virus from infected reservoir, currently thought to be fruit bats belonging to the family *Pteropodidae* [4]. However, no infectious EBOV nor complete RNA genome has ever been isolated from fruit bats. In the history of EVD, only Taï Forest virus (TAFV) has been associated with chimpanzees. Outbreaks of EVD are driven by human to human transmission through infected body fluids such as sweat, vomitus, blood and urine. EVD is characterised by initial non-specific symptoms such as sudden onset of fever, chills, and malaise accompanied by other signs such as myalgia, headache, nausea, abdominal pain, vomiting and diarrhoea [5]. The incubation period varies from 3-21days [6]. More characteristic haemorrhagic symptoms such as melena, haematochezia, epistaxis, haematemesis and bleeding from injection sites appear later in the course of the disease. Prolonged dehydration and development of multi-organ dysfunction leads to irreversible shock and eventually death. There are no licensed drugs and vaccines for the treatment of EVD although a number of vaccines and drugs are currently under development. This review attempts to summarise data from all of the published sources into a coherent explanation of the current knowledge about the structure and mechanism(s) of action of EBOV proteins. The paper also reviews the efficacy, concerns and mode of action of selected promising therapeutics and vaccines against EBOV. Suggestions on how to improve the efficacy of most anti-EBOV therapeutics and vaccines are also highlighted.

2. CLASSIFICATION AND STRUCTURE OF EBOLA VIRUSES

EBOV is a filamentous and pleomorphic negative sense RNA virus. The genus, *Ebolavirus* and two other genera, *Cuevavirus* and *Marburgvirus*, belong to the family *Filoviridae* and the order *Mononegavirales* [7,8]. Mononega viruses have a linear, nonsegmented, single-stranded enveloped RNA genome [7]. "They have a characteristic gene order: 3´-UTR —core protein genes—envelope protein genes—polymerase gene—5´-UTR" [2]. The latest filovirus taxonomy endorsed by the International Committee on Taxonomy of Viruses (ICTV) *Filoviridae* Study Group (2012-2014) and accepted by the (ICTV) are summarised in Table 1 [9,10].

All viruses in the genus *Ebolavirus* are pathogenic for humans except *Reston ebolavirus*, which is pathogenic for non-human primates [2]. The single stranded, non-segmented genome of EBOV is approximately 19kb in length [2].

Table 1. Latest taxonomy of filovirusesaccepted by ICTV

Order	Family	Genus	Species	Virus
Mononegavirales	*Filoviridae*	*Ebolavirus*	***Taï Forest ebolavirus* Virus**	**Taï Forest virus (TAFV)**
			Reston ebolavirus	Reston virus (RESTV)
			Sudan ebolavirus	Sudan virus (SUDV)
			Zaire ebolavirus	Ebola virus (EBOV)
			Bundibugyo ebolavirus	Bundibugyo virus (BDBV)
		Marburgvirus	Marburg marburgvirus	Marburg virus (MARV)
				Ravn virus (RAVV)
		Cuevavirus	*Lloviu cuevavirus*	Lloviu virus (LLOV)

The central core of the virus is made up of a single RNA genome and its adherent ribonucleocapsid complex. The complex is composed of nucleoprotein (NP), viral proteins (VP30 and VP35) and polymerase L [2,11]. GP forms trimeric spikes on the surface of EBOV that are anchored in the lipid bilayer. The genomic ends of EBOV known as ‘trailer’ (5’end) and leader (3’end) are non-transcribed and possess cis-acting signals vital for transcription and replication [12]. Transcription begins at the 3’ end to produce 7 structural proteins: NP, GP, P24, VP30, VP35, VP40, and polymerase L [2,12]. The seven genes that encode these proteins are arranged in a linear order, 3’-NP-VP35-VP40-GP-VP30-VP24-L-5’ [2].

3. NUCLEOPROTEIN AND RNA POLYMERASE L PROTEIN

Nucleoprotein (NP) is a principal constituent of the ribonucleocapsid complex together with RNA-dependent RNA polymerase (L), VP30 and VP35viral proteins [13-14]. The phosphorylated complex is responsible for viral transcription and replication [2]. In this complex, RNA dependent RNA polymerase L serves as an enzymatic component [2]. NP of EBOV is the largest nucleoprotein of all non-segmented negative-stranded RNA viruses with 739 amino acid residues [15]. It has a hydrophobic N-terminal half and a hydrophilic C-terminal half [16]. The C-terminal half is responsible for interaction with VP40 and the incorporation of NP into virions [17,18]. The N-terminal half is believed to be essential for RNA binding [15]. NP is O-glycosylated and sialylated [18]. These modifications are thought to be necessary for NP’s interaction with VP35 to support viral genome replication [19]. However, the type of O-glycosylation and sialylation has not been confirmed. EBOV NP is capable self-assembling (NP-NP interaction) to form helical tubes [17,19]. These helical tubes presumably act as the core of nucleocapsids.

4. GLYCOPROTEIN

GP is a type 1 transmembrane viral envelope protein. It is heavily glycosylated with *N-linked* and *O-linked* glycans accounting for nearly half of its molecular weight [20,21]. GP is expressed by two open reading frames (ORFs) through transcriptional RNA editing [14]. GP is responsible for receptor binding and viral entry into target cells [22]. It also augments the budding of virus like particles (VLPs) driven by the matrix protein, VP40 [17,18]. Maturation of EBOV GP involves posttranslational cleavage of a precursor (GP0) in the Golgi by a subtilisin-like endoprotease furinto yield two fragments, GP1 and GP2 [23-25]. Endoproteolytic cleavage of GP was previously considered an important determinant of EBOV virulence and in vitro infectivity [25]. However, other studies have proved that GP cleavage by furin is dispensable for in vitro virus replication and lethality of EBOV [23,24,26,27]. GP1 and GP2 are covalentlylinked via a disulphide bond between membrane anchored GP2 and the N-terminus of the extracellular GP1 subunit [22,25]. GP1 is required for interaction with receptors whereas the transmembrane associated GP2 is required for host cell membrane fusion [23-25].

GP1 subunit contains four domains: serine-threonine rich mucin-like domain (MLD), base, glycan cap, and receptor-binding domain (RBD) [2]. Base domain, glycan cap domain, and RBD make up the core of GP1. They are required for the expression and function of the prefusion glycoprotein [2]. The mucin domain encompasses approximately 150 amino acids and contains 4 N- and 13 *O-linked* glycosylation sites [28,29]. MLD appears to be essential for efficient interaction of GP with cell-surface C-type

lectins but deletion of this domain does not adversely affect the infectivity of pseudotyped virions *in vitro* [30,31]. The observed EBOV GP-mediated effects upon surface protein expression and cellular adhesion is due to the MLD of GP1 [31-32]. The base subdomain is made up of four interrupted sections of amino acid residues and forms a semi-circular hydrophobic surface that interacts with the internal fusion loop and heptad repeat region of GP2 [28]. Within the GP1 β2–β3 loop, proximal to the viral membrane end of the GP1 base is Cys53, which forms a disulfide bond to Cys609 of the GP2 subunit [28]. In contrast to the base subdomain, the glycan cap consists of a non-interrupted polypeptide chain that forms an α/β-dome over RBD and it has four *N-linked* oligosaccharides [28]. The glycan capis completely exposed on the outer surface of GP ΔmucΔtm but is not involved in any contacts at the trimer interface [28]. RBD is located between the base and glycan cap subdomains where itis surrounded by a heavy shield of glycans [33]. Disruption of this glycan shield results in increased cell entry, antibody sensitivity, and protease sensitivity but it is insufficient to allow virion binding to the intracellular receptor, Niemann-Pick C1 (NPC-1) [2,33]. A study by Ou et al. [30], found that two phenylalanines located at positions 88 and 159 of GP1 are critical for EBOV entry into cells. F88 is partially exposed on the GP1 surface next to the putative receptor binding site and the GP2 internal fusion loop where as F159 is deeply buried within a hydrophobic pocket [30]. F88 and F159 are surrounded by hydrophobic amino acids [30].

The transmembrane associated GP2 subunit is responsible for fusion of the viral and host cell membranes [34]. It contains a hydrophobic internal fusion loop and two heptad repeats (HR1 and HR2) which interact with the base subdomain of GP1 [28,34]. In the prefusion state, the internal fusion loop wraps around the outside of the GP trimer [28]. The hydrophobic amino acid sequences of this loop are bound by an antiparallel, disulfide linked β-scaffold, creating a β-sheet structure that resembles the GP structure of other viruses such as influenza viruses and flaviviruses [28,34-36]. HR1 is divided into four segments (A, B, C and D) [28,34]. HR1A and HR1B form anα-helix with a 40° bend at Thr565 and within this tight bend occurs an unusual 3–4–4–3 stutter rather than the normal 3–4 periodicity of heptad repeats [28,34]. This stutter may serve as a conformational switch [37].

5. NON-STRUCTURAL GLYCOPROTEINS

The 364-amino acid residuenon-structural soluble glycoprotein is directly synthesised from unedited mRNA of GP and is secreted from infected cells as a *disulfide-linked* homodimer [38,39] Post-translational proteolytic cleavage of pre-sGP by furin yields the mature sGP which has six predicted *N-linked* glycosylation sites, but only five sites are consistently glycosylated [38,40-41]. GP and sGP are expressed from a single glycoprotein gene and have identical 295 amino acid residues at their NH2-terminal ends but differ at the COOH termini by 69 amino acids [42]. Structurally, sGP is a parallel homodimer and is not in antiparallel orientation as first suggested by Volchkov et al. [40]. Soluble GP undergoes a post-translation modification known as C-mannosylation but the functional consequence of this modification is unknown [40]. The function of sGP remains unclear although a study by Bhattacharyya et al (2011), showed that in the absence of sGP, majority of full length GP synthesis is arrested in the endoplasmic reticulum(ER) or Golgi [27]. Soluble GP has also been shown to possess anti-inflammatory properties and its presence may modulate the expression of GP and cytopathology, allowing the virus to replicate for longer periods of time [41,43,44]. The ability of sGP to bind and inhibitearly activation of neutrophils through specific receptors (FcγRIIIb, CD16b) on neutrophils was challenged [45]. Neutralising and non-neutralising antibodies from sera of EVD survivors appear to preferentially recognise sGP over GP [21]. This is probably because sGP is released in large quantities than GP to serves as a decoy for EBOV specific neutralising and non-neutralising antibodies [21, 44].

Proteolytic processing of sGP at its C-terminus by furin yields a soluble heavily *O*-glycosylated, small, nonstructural, secreted protein termed Δ-peptide [46]. Δ-peptide is also released from infected cells [39]. In contrast to a mature sGP, Δ -peptide is retained in producer cells for longer periods of time prior to its release into the cell culture supernatant [46]. The function of Δ-peptide is not clear. The mRNA of the mature GP is generated by transcriptional editing resulting in the addition of a non-templated adenosine residue to a run of seven adenosine residues. Deletion of one adenosine or addition of two adenosines during transcriptional stuttering results in the expression of a third nonstructural, small sGP (ssGP) whose function is unknown

[38,41]. It shares 295 amino-terminal residues with sGP and GP and is secreted from cells in a monomeric form due to lack of the carboxyl-terminal part [38,42].

6. ROLE OF GLYCOPROTEINS IN THE UPTAKE OF EBOV INTO CELLS

The mechanisms utilised by EBOV to enter the host cell are not well understood. Clathrin- or caveolae-mediated endocytosis was proposed as one of the mechanisms of virus entry [47,48]. Other studies have gone further to suggest that internalisation of EBOV is dependent upon low pH and the endocytic enzymes cathepsin B and L [49-51]. It is thought that EBOV is internalised and sequestered within an endosome where it is exposed to a low-pH environment and crosses the endosomal membrane to reach the cytoplasm [49-51]. Conformational changes must occur in the viral GP spikes to enable its hydrophobic fusion domain to be introduced into the host cell membrane [25,51]. The conformational changes act as a switch that ignites the fusion of the virus to the host cell [25]. These conformational changes are caused by proteolytic cleavage of GP1by cathepsin B and L to yield an N-terminal fragment [25]. N-terminal fragmentis further digested by cathepsin to initiate membrane fusion by GP2, resulting in the release of the viral genome into the cytoplasm [34]. Internalisation of EBOV into the endosomes of cells leads to the removal of the C terminus of GP1 by either of the protease enzymes or both. Other studies have suggested that EBOV is taken up into host cells via macropinocytosis in a glycoprotein dependant manner. Nanbo et al. [52] found that internalisation of EBOV is independent of clathrinor caveolae-mediated endocytosis, but co-localise with sorting nexin (SNX5). Sorting nexin (SNX) 5 comprises a family of peripheral membrane proteins and serve as markers of macropinocytosis specific endosomes known as macropinosomes [52]. Additionally, it was found that the internalisation of EBOV virions accelerates the uptake of a macropinocytosis-specific cargo, and was associated with plasma membrane ruffling [52]. The mechanism was reliant on cellular GTPases and kinases that are involved in macropinocytosis [52].

7. VIRAL PROTEIN 30 (VP30)

VP30 is one of the EBOV structural proteins that form the ribonucleocapsid complex [53]. It is a strongly acidophilicprotein and its presence in the complex is essential for initial transcription in newly infected cells [54]. The role of VP30 in EBOV replication is limited to stabilizing nascent mRNA [55]. In other words, VP30 is important for initiation and re-initiation of EBOV transcription but is inessential for viral genome replication [56,57]. Formation of an RNA secondary structure upstream of the transcriptional start site of the first gene of EBOV is the driving force behind the function of the heavily phosphorylated VP30 as a transcription activator [58,59]. This posttranslational modification has been shown to regulate the activity of VP30 during transcription [60]. The proposed function of VP30 as a transcription anti-termination factor has not yet been substantiated [55,58]. Sequence analysis of filoviral VP30 protein revealed that it containsa motif resembling that of an unconventionalzinc-binding Cys3-His motif that was initially described for Nup475, a mammalian nuclear protein [55]. Cys3-His motif has been shown to have functional relevance for viral growth [61]. VP30 is phosphorylated at 2 serine clusters (amino acids 29–31and 42–46) [57,60].

The molecular mechanisms by which VP30 activates viral transcription are obscure. John et al. [53], found that VP30 directly interacts with RNA and promotes transcription through a region within its N terminus. This region has sequence features that characterise intrinsically disordered regions and a putative RNA binding site [53,62]. The key amino acids that support RNA binding activity were mapped to a short arginine rich amino acid stretch (residues 26 to 40) of VP30 [53]. It has also been suggested that the function of VP30 as a transcription activator is exerted by a homooligomer of VP30, which requires a hydrophobic stretch of leucine residues in the N terminus [54,63]. Furthermore, Hartlieb et al. (2006), provided evidence that VP30 contains a second homooligomerization domain which is composed of the C-terminal half of VP30 [54]. Additionally, transcription activation by VP30 strongly relies on the phosphorylative state of VP30 [57,60]. None or weakly phosphorylated VP30 activates transcription while fully phosphorylated VP30 acts as an efficient inhibitor [57,60].

8. VIRAL PROTEIN 35 (VP35)

VP35 is involved in the assembly of the EBOV ribonucleocapsid complex. It plays an important role in viral transcription, replication and suppression of host IFN immune responses (2)

(Fig. 1). VP35 inhibits host interferon (IFN) responses by binding double stranded RNA (dsRNA) [64]. This function is embedded in its 130 amino acid C-terminalds RNA binding domain (RBD) [65]. The N-terminal half of RBD contains anα-helical subdomain whereas the C-terminal half contains a three-stranded mixed β sheet with a conserved basic patch [66-70]. The VP35 of EBOV binds the ends of dsRNA while that from MARV primarily binds in the middle of dsRNA [71]. Using VP35 structures from RESTV, Kimberlin et al. [68], showed that VP35 RBD forms an asymmetric dimer with two distinctive modes of RNA binding: one monomer, termed the "endcappingmolecule", binds the terminal bases of dsRNA while another monomer termed the "backbone-bindingmolecule", dimerises with the end-capping molecule and binds along the neighbouring sugar-phosphate backbone [68]. Building on the findings by Kimberlin et al. [68], Bale et al. [65] demonstrated that VP35 coats the dsRNA central backbone of EBOV to shield it from recognition by virus specific host pattern recognition receptors (PRRs) and prevent induction of subsequent innate and adaptive immune responses [65, 68]. VP35 inhibits virus-induced activation of type I IFN promoters by targeting a transcription factor known as IFN regulation factor 3 (IRF-3) [72,73]. However, activation of IFN promoters through exogenous interferons is not regulated by VP35 [73].

The ability of VP35 to block the expression of type 1 IFNs has been traced to its C-terminal dsRNA-binding domain, also known as IFN inhibitory domain (IID) [64,74]. Studies by Cardenas et al. (2006) and Hartman et al. [75] cemented the role of VP35 as a suppressor of early innate immunity by demonstrating that VP35 mutated viruses are incapable of blocking the IFN-dependent induction of antiviral factors [64,75]. Other mutational studies have shown that the N-terminal coiled domain in VP35 mediates homo-oligomerisation, which is essential for IFN antagonism exhibited by the C-terminal half of VP35 [76]. VP35 uses other mechanisms to suppress innate immune responses. Luthra et al. [77], demonstrated that VP35 inhibits PACT-induced RIG-I ATPase activity [77]. Interactions between PACT and VP35 impairs the association between VP35 and the viral polymerase L, resulting in diminished viral RNA synthesis [77]. Generally, cells that are PACT-deficient are defective in IFN induction and are insensitive to the function of VP35 [77]. Other studies suggest that VP35 may inhibit the RIG-I pathway by acting as a decoy substrate for the cellular kinases IKKε and TBK-1 and through interaction with the Sumoylation machinery [78,79]. Additionally, VP35 inhibits antiviral responses induced by type 1 IFNs by interfering with the pathway regulated by IFN-inducible dsRNA-dependent protein kinase (PKR) [80]. Automatic phosphorylation of PKR is disrupted in cells expressing VP35 resulting in the inhibition PKR activation [80]. However, work by Schumann et al. [81], indicated that mutations in the C-terminal dsRNA-binding domain of VP35 are sufficient to rescue the blockade to PKR activation imposed by VP35.

VP35 is also an efficient suppressor of RNA interference (RNAi) [82,83]. RNAi is a potent cellular defence mechanism that allows host cells to detect and destroy viral RNAs in order to combat viral infection [82]. The mechanisms through which VP35 suppresses RNAi are still obscure. Haasnoot et al. [83], suggested that VP35 shields dsRNA from recognition by a central molecule of the RNAi pathway known as dicer [83]. Although Haasnoot et al. [83] claimed that VP35 suppresses RNAi, the mechanism he and others suggested is not a typical mode of suppression. The correct terminology should have been 'shielding'. Moreover, other studies refuted mechanism suggested by Haasnoot et al. [83] by demonstrating that VP35 does not interact directly with dicer, but interacts with two dsRNA-binding proteins (HIV-1 trans-activation response RNA-binding protein and protein activator of PKR(PACT)) which are part of the RISC complex [84]. Innate immune responses against viral infections are primarily mediated through macrophages and dendritic cells (DCs) which link innate and adaptive immunity [85-87]. Immature DCs reside in almost all peripheral tissues. In response to viral infections and toll like receptor-4 (TLR-4) signalling, immature DCs migrate to lymph nodes where they undergo maturation and possess relatively higher levels of co-stimulatory molecules [88-90]. However, VP35 impairs the maturation of DCs by replicating efficiently in immature DCs without inducing the expression of cytokine, leading to impaired T-cell proliferation [87,89,91]. Expression of EBOV VP35 in mouse immature DCs prevents virus-stimulated expression of CD40, CD80, CD86 and MHC class II and suppresses induction of cytokines such as interleukin (IL)-6, IL-12, tumour necrosis factor-α (TNF-α)and type 1 IFNs (IFN-α/β) [89]. VP35's activities to block DC maturation and function contributes to the virulent nature of Ebola viruses.

9. VIRAL PROTEIN 24 (VP24)

VP24 is the smallest EBOV structural protein [92]. It plays a role in the assembly of a fully functional ribonucleocapsid complex and contributes to the budding of virus-like particles (VLPs) [92]. As a component of the ribonucleocapsid complex, it is expected that VP24 plays a role in EBOV transcription and replication. However, Watanabe et al. [93], reported that VP24 reduces transcription and replication of the EBOV genome. VP24 has a single domain, α/β structure, who secrystal shape resembles a triangular pyramidal fold upon which sites required for virulence and for STAT1 binding have been mapped [94]. The three sides of the VP24 pyramid are termed Faces 1, 2 and 3 [94]. The putative STAT1-binding site on VP24 lies in the conserved region of Face 3 and is distinct from the site proposed to interact with karyopherin α1 [94].

VP24 is capable of inhibiting host IFN response to viral infections by blocking the nuclear translocation of the transcription factor STAT1 [95] (Fig. 1). STAT1 is a key component of the IFN-induced signalling pathway that controls the expression of IFN stimulating genes (ISGs). Using VP24 protein, EBOV has been shown to indirectly affect the translocation mechanism of STAT1 by binding to karyopherin α1 and blocking it from transporting activated phosphorylated STAT-1 (PY-STAT1) complexes into the nucleus of host cells [95-96]. It also disrupts the recognition of the PY-STAT1 nuclear localisation signal (NLS) by karyopherin α, thereby blocking the translocation of PY-STAT1 into the nucleus [95-96]. Furthermore, VP24

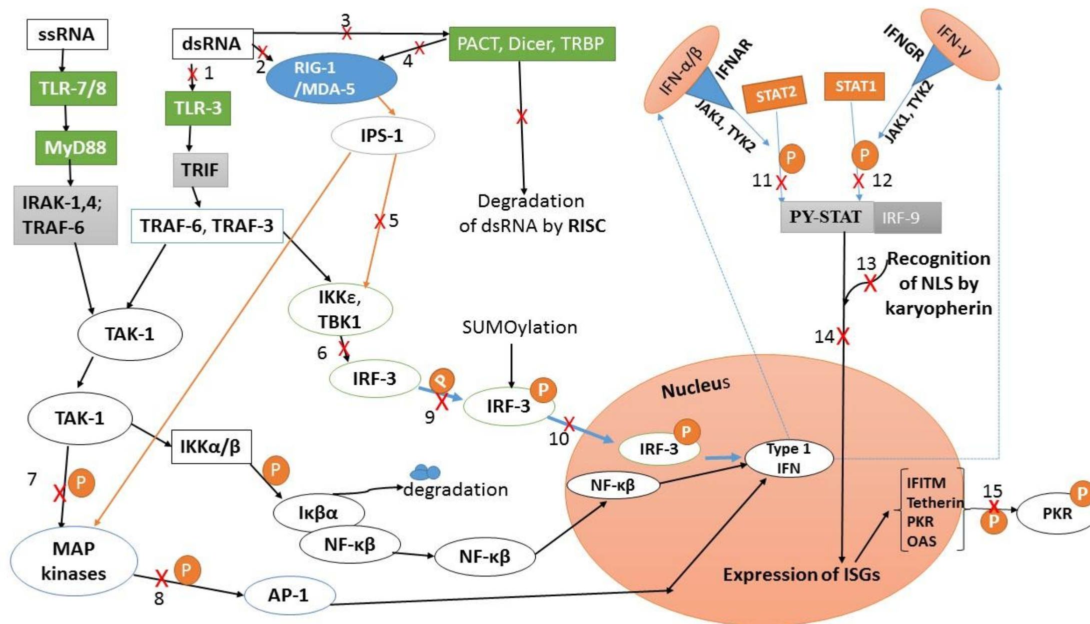

Fig. 1. Interference of EBOV VP35 and VP24 with the expression of IFN and ISGs

1,2,3VP35 coats the dsRNA central backbone of EBOV to shield it from recognition by virus specific host pattern recognition receptors (PRRs) and degradation by the host RNA-induced silencing complex (RISC). 4VP35 inhibits PACT-induced RIG-I ATPase activity. 5,6 VP35 impairs the interaction between IKKε-IRF-3 and IKKε-TBK1. 7,8 VP35 inhibits the phosphorylation of p38 in the MAP kinase pathway. 9,10 VP35 induces SUMOylation of IRF-3 and interferes with the phosphorylation and nuclear translocation of IRF-3. 11,12 VP24 and VP35 blocks the phosphorylation of STAT1/2. 13 VP24 disrupts the recognition of the PY-STAT1 nuclear localisation signal (NLS) by karyopherin α. 14 VP24 blocks the translocation of PY-STAT/IRF-9 complex into the nucleus. 15Autophosphorylation and activation of PKR is blocked by VP35.TRIF, TIR domain-containing adaptor-inducing IFN-b; MyD88, myeloid differentiation primary response protein 88; TRAF6, tumour necrosis factor receptor-associated factor 6; IPS-1, IFN-b promoter stimulator 1; TBK1, tank-binding kinase 1; IRAK, interleukin-1 receptor-associated kinase; IFITM, IFN-induced transmembrane protein; TRBP, trans-activation response RNA-binding protein.

binds to a stretch of amino acids known as armadillo repeat 10 (ARM10) that encompasses part of the binding site for PY-STAT1 within karyopherin α1 [95-97]. Targeting the ARM10 repeat of karyopherin-α by VP24 may block binding of PY-STAT1 because of competitive inhibition. Amino acid residues 42 and 142–146 in VP24 are critical for the inhibition of IFN-β-induced signalling and accumulation of PY-STAT in the nucleus [98]. VP24 mutants with amino acid exchanges at these positions neitherbind karyopherin-α nor inhibit IFN-β induced signalling indicating that binding of VP24 to karyopherin-αis essential for IFN antagonism [98]. EBOV VP24 is well known for its IFN antagonist activities through the JAK-STAT pathway. However, recent data suggests that VP24also interferes with the p38 MAP kinase pathway by blocking IFN-β–stimulated phosphorylation of p38-α [99]. Phosphorylated p38 is responsible for initiating the phosphorylation of downstream transcription factors that play key roles in IFN responses [99].

10. VIRAL PROTEIN 40 (VP40)

VP40 is the major matrix protein of EBOV representing about 40% of molecular weight [100]. It is located beneath the viral lipid membrane, where it probably helps to maintain the structural integrity of the virus [101-102]. It also plays an essential role in viral replication, assembly and budding [2,103]. VP40 virus like particle (VLP) budding is driven by viral L domains located in its N-terminus [101-102]. Through these domains, VP40 interacts with cellular factors such as the Nedd4, ubiquitin ligase, and TSG101leading to the release of VLPs [104-106]. VP40 is capable of producing VLPs in the absence of other EBOV proteins when expressed in human cells due to its RNA binding and oligomerisation properties [100, 107]. However, co-expression of VP40 with other EBOV proteins enhancesthe budding of VLPs with little effects on VLP morphology [11,108,109].

The crystal structure of VP40 consists of two distinct domains, N-terminal domain (NTD) and C-terminal domain (CTD), connected by a flexible linker [110-11]. NTD regulates dimerisation while CTD has been shown to mediate membrane binding and oligomerisation [100-111]. Three immuno-dominant regions of EBOV VP40 located in CTD (amino acid residues 189–207, 213–235 and 289–319) reportedly play a critical role in virus assembly and are a target of host humoral responses [100]. EBOV IgG+ seraobtained from asymptomatic individuals during the 1995 Gabon outbreaks of EVD reacted strongly to these regions than to GP, NP or VP35 [100]. This study by Becquart et al. [100], strongly suggests that humoral responses directed against the three key epitopes/domains of VP40 may contribute to protecting humans against EVD. The mechanistic details of VP40 membrane binding vital for viral releaseare still obscure. Phosphatidylserine, an anionic lipid present in the inner leaflet of the plasma membrane of human cellshas been shown to be essential for VP40 CTD plasma membrane interactions [112-114]. A cationic patch in the CTD is believed to mediate association with anionic membranes while a hydrophobic loop drives the penetration of CTD into the hydrocarbon core of the plasma membrane lipid bilayer causing distinct changes [112-114]. This is an essential step in viral egress becausehydrophobic residues that penetrate deeply into the plasma membrane are vital for plasma membrane localisation, formation and release of VLPs from host cells [114]. VP40 also mediates viral transcription through formation of an RNA binding octameric ring and interacts with actin, microtubules, Ras GTPase-activating-like protein (IQGAP1), COPII protein and Sec24C [115-120].

11. POTENTIAL VACCINE PLATFORMS AGAINST EBOV

Overexpression of genes that encode EBOV proteins is the current primary approach to vaccine development. This approach aims at producing sufficient proteins that elicit potent T and B cell-mediated immunity against EBOV. A number of viral and non-viral vectors are utilised to deliver genes for these antigens (EBOV proteins) in order to stimulate strong adaptive immune responses (Table 2). The first vaccine platform utilising a vector was a recombinant adenovirus type 5 (rAd5) vector expressing EBOV GP and NP [121]. A single intramuscular (IM) injection of this vaccine after three priming dosesof plasmid DNA encoding EBOV GP, NP, SUDV GP, and TAFV GP fully protected cynomolgus macaques against lethal EBOV challenge [121]. This approach boosted the circulating levels of anti-GP neutralising antibodies and $CD4^+/CD8^+$ T cell immune responses against EBOV. Subsequent studies have shown that a single IM injection of the adenovirus vector expressing GP alone could protect animals from EBOV infections [122,123]. Ad5-based vaccine has been refined further by

optimising the GP expression cassette resulting in a 100 fold reduction in dosage [124]. Earlier concerns about pre-existing immunity (PEI) against rAD5-based vaccines have been addressed through the use of chimpanzee based adenovirus vaccines and use of other adenovirus serotypes and use of different delivery routes [125-127].

Other vaccine platforms such as recombinant vesicular stomatitis virus (rVSV) utilise live attenuated recombinant viruses bearing the wild type EBOV GP. A single dose of rVSV fully protected guinea pigs, mice and non-human primates (NHPs) from a lethal dose of EBOV [128-130]. However, an inactivated form of VSV (gamma-irradiated) did not offer protection to animals against an EBOV lethal challenge, indicating that replication is key to the potency of this vaccine [131]. Another live-attenuated vaccine is the expressing EBOV GP alone or together with NP [132,133]. Issues of pre-existing immunity to this vector were a major limitation but development of chimeric human parainfluenza virus 3 vectors expressing EBOV GP has reduced the effects of PEI [134]. Other vaccine platforms utilising virus vectors expressing EBOV GP include Venezuelan Equine Encephalitis virus replicon, rabies virus based vaccines and the rare recombinant adenovirus serotypes (rAd26 prime /rAd35 boost) [135]. These platforms have been found to confer protection to NHPs after a lethal EBOV challenge even though they all need boost immunisation [135].

VLP vaccines are also promising vaccine platforms. VLPs depend on the properties of VP40 to drive budding of filamentous particles after co-expression in mammalian and insect cells [17]. VLPs can also be engineered to incorporate other EBOV proteins such GP, VP24, and NP [17,136-138]. These spherical to filamentous structures are 400–600 nm with a diameter of 70–100 nm and they resemble authentic filoviruses [17,136-138]. VLPs are capable of inducing maturation and activation of murine and human DCs, inducing increased surface expression of CD80, CD83, CD86, major histocompatibility complex (MHC II and MHC I), and induced secretion of pro-inflammatory cytokines such as IL-8, tumor necrosis factor-α (TNF-α), and macrophage inflammatory protein–1α [91,138-140] VLP vaccines use particles containing two proteins (GP and VP40) or three proteins (GP and VP40 plus NP). Recent research has demonstrated that VLP vaccines containing GP, VP40 and NP confers full homologous protection against EBOV in a prophylactic setting in NHPs [138]. Currently, the VLP based vaccine candidate against filoviruses is an enveloped VLP with GP spikes on the surface of the lipid bilayer holding VP40 [141].

12. POTENTIAL DRUGS AND MONOCLONAL ANTIBODIES AGAINST EBOV

A number of potential drugs and antibody cocktails targeting different stages of EBOV life cycle and sequences within structural proteins to block virus entry, transcription and replication are currently under development (Fig. 2).Small

Table 2. Current vaccine platforms tested in NHPs, mice and guinea pigs

Vaccine platform	Target in vaccine	Administration route	Ref
Human parainfluenza virus type 3	GP, GP+NP	Intranasal, intratracheal	[132-134]
Recombinant adenovirus serotype 5	GP, GP+NP	Intramuscular, mucosal	[121-123]
Adenovirus serotypes (rAd26 prime /rAd35 boost)	GP	Intramuscular, mucosal	[125-127]
DNA/rAd5(Prime/Boost)	GP+NP	Intramuscular, mucosal	[121, 122]
Vesicular stomatitis virus	GP	Intramuscular, intranasal, oral route	[142-144]
Virus-like particles (VLPs)	GP+NP+VP40	Intramuscular,	[138-139,141]
Venezuelan Equine Encephalitis virus replicon	GP	Intramuscular	[145]

interfering RNAs (siRNAs) are one of the most promising anti-EBOV compounds that bind to sequences within EBOV RNA polymerase L, VP24, and VP35 regions to block viral transcription and replication [146]. They are antisense oligonucleotides that are complementary to sequences in the EBOV genome [146]. Geisbert et al. [146] identified three specific siRNA compounds that bind to polymerase L (EK-1), VP24 (VP-24-1160), and VP35 (VP-35-855). Intravenous administration of these compounds formulated as stable nucleic acid-lipid particles (SNALPs; LNP/siRNA: TKM-Ebola) protected 66%of NHPs against a lethal dose of EBOV [146]. However, a seven-day regimen using the same compounds provided full protection to NHPs although an increase in serum aspartate aminotransferase was observed in all animals [146]. Apart from the potential damages to the liver and kidneys, siRNAs also require multiple doses (11 doses) to be effective [146]. Therefore, the greatest challenge going forward is to reduce the number of doses especially in the light of the increased serum aspartate aminotransferase associated with the use of these compounds.

Phosphorylation of EBOV VP40 by an enzyme known as c-Abl1 is essential for transport of the nucleocapsid complex to the host cell membrane and release of mature virions from the cell [147]. Compounds such as Imatinib and Nilotinib have been found to block the phosphorylative activities of the c-Abl1 enzyme resulting in limited release of complete infectious EBOV virions in culture medium [147]. Although, Imatinib (Gleevec®) and nilotinib (Tasigna®) are approved treatments options for leukaemia in humans, their role in EBOV treatment has not investigated to the fullest [147]. The fact that they are anticancer drugs make them unsuitable for treating EVD because of their immunosuppressive nature. EVD suppresses the host immune system hence drugs that support immune stimulation have a higher percentage of controlling the virus. It is a fact that there are so many compounds out there that can inhibit replication of EBOV but that does not make them potential treatment options for EVD. Imatinib and Nilotinib fall this this category of compounds.

Another potential post exposure therapeutic option against EBOV involves the use of phosphorodiamidate morpholino oligomers (PMOs). PMOs are third generation synthetic antisense oligonucleotides [148]. They block the replication of EBOV by arresting translation and mRNA processing within polymerase L, VP24 and VP35regions through steric hindrance [148]. A study by Warren et al. [148], showed that administration of positively charged PMOs (AVI-6002) 30-60 minutes post infection, protected five out of eight macaques infected with a lethal dose of EBOV. PMOs are currently under phase I clinical trials to establish their efficacy, safety and timings [148]. The main weakness with PMOs are the short timeframes within which they are effective to offer post exposure protection against EBOV infection and the need for multiple doses. Thus, more research is required to increase the time frames from minutes to weeks and to reduce the number of doses required for efficacy. Currently seven doses at 2 mg/kg body weight are required for the drug to be effective in NHPs [148].

The current outbreak of EVD in West Africa saw the first use of antibody based treatment options in selected individuals with varying results. IgG positive serum from EVD survivors was also used to treat certain individuals. However, the effectiveness of monoclonal antibody based therapies in humans is unclear. Over the last decade, research has been ongoing to determine the effectiveness of monoclonal antibodies in NHPs exposed to lethal doses of EBOV. A combination of three EBOV GP-specific murine monoclonal antibodies (1H3, 2G4, and 4G7) known as ZMAb protected 100% and 50% of NHPs from a lethal dose of EBOV, 24 and 48 hours after infection, respectively [149,150]. In a subsequent experiment (10-13 weeks later), the surviving animals were challenged with another lethal dose of EBOV to evaluate whether the initial challenge was sufficient to protect the animals against subsequent exposure to EBOV [151]. The data indicated that a robust immune response was generated resulting in sustained protection against the second lethal dose of EBOV [151]. Another combination of chimeric human-mouse monoclonal antibodies (c13C6, h-13F6, and c6D8) produced in Chinese hamster ovary and whole plant cells was also able to protect NHPs from a lethal challenge of EBOV, 24-48 hours post exposure [152]. This cocktail of monoclonal antibodies is known as MB-003. In a separate study MB003 also conferred post exposure protection to 43% of monkeys, 5 days after administration of a lethal challenge of EBOV [153]. More recently, a combination of ZMAb and MB-003 called ZMapp, has been found to be more effective against EBOV in NHPs than any of the two individual cocktails of monoclonal antibodies [154]. ZMapp fully protects animals 5

days post-EBOV infection. An earlier combination of the chimeric mAbs, ch133 and ch226, has also been found to be effective against EBOV in NHPs [155]. The chimeric nature of antibodies described in this review has been described [156,157].

Other antibody based therapies that combined monoclonal antibodies with adenovirus-vectored IFN-α (Ad-IFN) provided 75% protection to cynomolgus macaques and 100% protection to rhesus macaques, 3 days after detection of viraemia [158]. In a separate study, the combination of MAbs with an adenovirus vectored IFN (DEF201) was also evaluated in guinea pigsexposed to a lethal dose of EBOV [159]. DEF201 alone extended the mean time to death significantly but failed to provide survival to the animals [159]. Unlike previous studies, only 33% of animals treated with MAbs alone survived the infection. Interestingly, all guinea pigs that received both DEF201 and MAbs 3 days post infection survived the lethal EBOV infection whereas only 50% of guinea pigs that received treatment 4 days post infection survived [159]. Overall, both studies by Qiu et al. [151], suggest that treatment regimens that include cocktails of monoclonal antibodies and IFN-α/β have a broader spectrum of activity and may help circumvent the IFN suppressive activities of EBOV while stimulating maturation of DCs and activation of tetherin. In addition, combining different neutralising antibodies into cocktails make them more effective against EBOV even in the absence of IFNs. This is probably because each neutralising antibody binds to specific regions of GP to neutralise the virus. For example, 1H3 and c13C6 bind to GP_1 and sGP, whereas h-13F6 and c6D8 bind to linear epitopes of MLD, one of the four domains of GP_1 [155-157]. These findings together with a study that demonstrated that IgG positive sera from symptomatic and asymptomatic survivors of EVD react differently to different EBOV proteins are a positive milestone [100]. We are therefore convinced that a cocktail of sera from symptomatic and asymptomatic survivors of EVD may be more effective in the treatment EVD during an outbreak of the disease. However, this conviction needs more research to determine the common types of neutralising antibodies present in both groups of survivors.

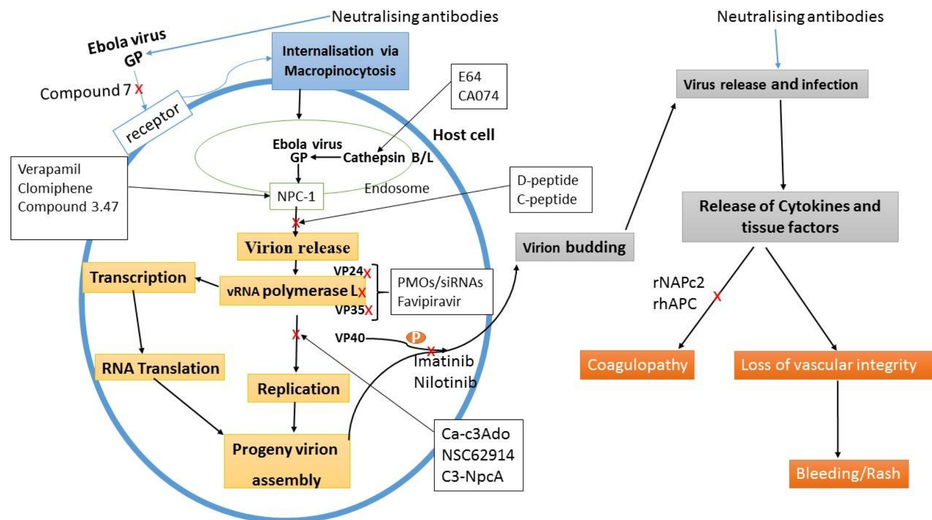

Fig. 2. Replication cycle of EBOV and the sites of action of current potential anti-EBOV compounds and neutralising antibodies

Small interfering RNAs (siRNAs) and phosphorodiamidate morpholino oligomers (AVI-6002) bind to sequences within the EBOV polymerase L, VP24, and VP35 regions to inhibit viral transcription and replication. Favipiravir also inhibits polymerase L. Imatinib and nilotinib block the transport of the nucleocapsid complex to the host cell membrane and release of mature virions from the cell by inhibiting phosphorylation of VP40. Compound 7, a benzodiazepine derivative interferes with EBOV entry. Verapamil, Clomiphene, and Compound 3.47 inhibits EBOV release from endosomes by binding to Niemann-Pick C1. D-peptide and C-peptide also prevent EBOV release from the endosome. Cysteine protease inhibitors, E64 and CA074 block endosomal cellular cathepsin B and L from processing GP resulting in the inhibition of EBOV release from endosome. Recombinant nematode anticoagulant protein c2 (rNAPc2) and rhAPC reduce abnormal clotting mechanisms during EBOV infections. Ca-c3Ado, NSC62914 and C3-NpcA directly block the replication of EBOV. Neutralising antibodies target EBOV GP.

High through put screening systems has led to the discovery of many compounds that are capable of controlling EBOV. Such compounds include a benzodiazepine derivative known as compound 7 [160]. This compound is thought to interfere with EBOV entry. The exact mechanism used by this compound to block EBOV cell entry is unknown but it probably interferes with the $GP_{1,2}$ trimer. More research is needed not only to determine how compound 7 blocks virus entry but also the doses, safety and time frames within which it is effective against EBOV. Another molecule known as Compound 3.47, derived from benzylpiperazine adamantine diamide (2-((3r, 5r, 7r)-adamantan-1-yl)-N-(2-(4-benzylpiperazin- 1-yl)-2-oxoethyl) acetamide), inhibits EBOV release from endosomes by binding to Niemann-Pick C1 [161]. Although it is able to inhibit EBOV release from endosomes, compound 3.47 is not safe to use in EVD patients. It is known to mediate a devastating neurodegenerative condition, called Niemann-Pick Type C disease [169]. Other compounds such as EBOV D-peptide conjugated to the Fc region of a human IgG1 antibody and the endosome targeting C-peptide derived from native C-terminal heptad repeat regions of EBOV GP2 conjugated to the arginine rich sequence of HIV-1 trans-activator of transcription also prevent EBOV release from the endosome [162]. However, nothing is known about the safety, dosage and the mechanism it utilises to control EBOV release. Cysteine protease inhibitors, E64 and CA074, have also been shown to block endosomal cellular cathepsin B and L from processing GP resulting in the inhibition of EBOV release from endosome [51]. These compounds have only been tried in vitro and mice hence, their potential effects in NHPs are unknown. Moreover, the dosage and time frames are unknown. There is still a lot of work that needs to be done before these drugs can be used to treat EVD. Other anti-EBOV compounds responsible for replication of EBOV include s-adenosyl-homocysteine (SAH) hydrolase inhibitors 3-deazaneplanocin A (c3-NpcA), carbocyclic 3-deazaadenosine (Ca-c3 Ado) and NSC62914 (an antioxidant that acts as a scavenger of reactive oxygen species) [163-165]. C3-NpcA andCa-c3 Adorequire higher doses (80mg/kg) in rodents and can control the host immune system making them less suitable for treating EBOV which is also immunosuppressive. However, more research is needed to evaluate the impact of the immune controlling mechanisms of these two drugs in cells infected with wild type EBOV and control cells.

Clotting disorders or coagulopathies occur in many EVD patients. This has been attributed to over production of pro-coagulant tissue factors resulting in haemorrhagic symptoms and a reduction in circulating protein C. Exogenous Factor VIIa or tissue factor inhibitor [recombinant nematode anticoagulant protein c2 (rNAPc2)] and recombinant human activated protein C (rhAPC) have shown potential to control abnormal coagulation [166]. However, conflicting reports from phase two clinical trials in USA cast doubt on these two drugs [166-167]. Consequently, rhAPC was withdrawn from the market in 2011 [167]. Other potent anti-EBOV compounds include favipiravir, clomiphene and Verapamil. Favipiravir is capable of suppressing the replication of EBOV in cell culture by binding to RNA polymerase L [168]. A study by Oestereich et al. [168], showed that favipiravir administered 6 days post EBOV infection fully protects mice from a lethal dose of EBOV. Similar studies conducted by Smither et al. [169], proved the efficacy of favipiravir against EBOV in mice. Favipiravir may be effective in mice but nothing is known about its potency, dosage, and safety in NHPs. Clomiphene and Verapamil block the entry of EBOV indirectly via NPC-1 by targeting other endosomal/lysosomal proteins involved in the cholesterol uptake pathway whose function may be regulated by NPC1 [170].

13. FUTURE PERSPECTIVES

13.1 Development of More Effective Therapeutics and Vaccines

Each EBOV protein has its own unique mechanism(s) of contributing to the ability of the virus to suppress or evade the host immune system. However, VP24 and VP35 are responsible for the majority of immune suppression seen in EVD. Therefore any potential anti-EBOV therapeutics and vaccines that target these two proteins may hold the key to effective treatment of EVD. Currently, most potential vaccines such as rHPIV3, rAd5, rVSV, Venezuelan equine encephalitis virus replicon, and rabies virus based vaccines have been developed with the goal of stimulating B and T-cell immune responses against EBOV GP alone. Vaccines that solely focus on the surface GP to induce anti-viral immunity are weakened by the ability of EBOV to secret sGP, a non-structural protein that serves as a decoy to neutralising antibodies. Moreover, it has been suggested that infection of host cells by EBOV is enhanced by the complement protein (C1q) and neutralising

antibodies to the viral GP [171]. Other studies have also suggested that neutralising antibodies from EVD survivors have a preference for sGP than GP although this may not be correct because sGP is released in large quantities as a decoy and more antibodies are be expected to bind sGP than GP [172]. In a more striking study, EBOV IgG positive sera obtained from asymptomatic individuals during outbreaks of EVDin Gabon in 1995 reacted strongly to VP40 than to GP and NP while IgG^+ sera from most survivors reacted mainly with GP peptides [100]. This study suggested that neutralising antibody immunity against GP may help one to survive EVD but preventing the virus from causing disease may require a stronger humoral immunity against VP40. There is enough evidence that vaccines that are aimed at eliciting immunity against GP alone may not beoverall solutions to fighting EVD. However, their weaknesses may be evaded by developing treatment regimens that include IFNs, cocktail of neutralising antibodies and other anti-EBOV compounds.VLP based vaccines are promising alternatives to virus vectored vaccines because they are capable of inducing humoral and cell mediated immunity against EBOV GP, VP40, and NP [138,141]. Their potency and spectrum may be increased by engineering them to incorporate VP24 and VP35.

PMOs and siRNAs are also promising therapeutics against EBOV. They target mRNA sequences within the regions of VP40 and VP35 to halt EBOV transcription and replication [146, 148]. They are capable of significantly blocking VP24 and VP35 from inhibiting the maturation of DCs, mitogen activated pathways, and other IFN signalling pathways [146,148]. However, they are only effective when administered approximately 30-60 minutes post EBOV infection and require multiple doses (in the case of siRNAs) [146,148]. In the natural world, it is not possible to determine when a person gets infected with EBOV. Thus, the main challenge going forward is to improve the timings of these drugs so that they can become effective several days after infection with EBOV. Otherwise, their promising efficacy will be limited to laboratory conditions. Additionally, administering these compounds as supportive therapies to vaccinations and antibody concoctions may make them more effective but that will not override the challenge posed by timings.

IFNs play critical roles in direct antiviral immunity as well as linking innate and adaptive immune responses. Through VP35 and V24, EBOV has devised mechanisms to inhibit all the IFN signalling pathways. However, activation of IFN promoters through exogenous IFNs is not regulated by these proteins [73]. Therefore, use of exogenous IFNs may be crucial to circumventing the ability of VP24 and VP35to inhibit IFN expression and maturation of DCs [89, 173]. We therefore suggest that IFNs must be part of treatment combinations against EVD. Our suggestion is based on the observed failure of EBOV proteins to inhibit and regulate the activation of IFN promoters through exogenous IFNs. This is in addition to studies by Qui et al. (2013) that showed that Monoclonal antibodies combined with adenovirus-vectored IFN significantly extend the treatment window in EBOV infected guinea pigs and NHPs [158,159] Exogenous IFNs may also induce the expression of another antiviral protein known as tetherin. Tetherin, also known as BST-2 or CD317, is a type I IFN induced, transmembrane protein. Blanchet et al. [174] demonstrated that expression of tetherin in immature myeloid and monocyte derived DCs is upregulated by IFN-α treatment. Kaletsky and others (2009), demonstrated that tetherin inhibitsthe release of EBOV from the surface of infected cells [175]. Tetherin is a potent anti-viral protein but its role in inhibiting replication and transcription of EBOV needs further investigations.

Based on our analysis of the structure and mechanisms of action of EBOV proteins and the various promising vaccines and therapeutics against EBOV, we have realised that many of the current promising therapeutics and vaccines are incapable of fully controlling EBOV infection single handedly. Therefore, combining different therapeutics and creating regimens that involve the use of both therapeutic concoctions and vaccines may prove to be more effective against EVD.

13.2 Fruit Bats and Insectivorous Free Tailed Bats in Ebola Virus Disease

Over the past 20 years, research has been focused on detecting EBOV antibodies in fruit batsbut no infectious virus has ever been isolated from these animals [2,176]. Therefore, the search for natural reservoirs of EBOV must continue and should be extended to other wild animals. Over a decade ago, rodents were thought to be potential reservoirs of EBOV but no other papers have been published to confirm the initial findings [177]. The recent discovery of

insectivorous free-tailed bats (*Mops condylurus*) in guinea as possible sources of EBOV has expanded the range of potential natural reservoirs of the virus [178]. However, there is a possibility that both insectivorous bats and fruit bats are exposed to the virus through the food chain. Therefore, the types of insects consumed by these insectivorous free tailed bats in the region where the EVD originated from must be investigated to determine whether they are the actual natural reservoirs of the virus. Despite the significant progress in the search for EBOV reservoirs, virtually nothing is known about the virus-host interactions yet bats are reservoir hosts to several other human viral pathogens such as lyssaviruses, henipaviruses and coronaviruses. Biochemical and genetic evidence suggests that bats use same systems for surveillance of viral threats like other mammals [179-182]. With regard to the noncoding RNAs and immunological genes, bats are similar, but not identical, to other mammals [183-185]. Most of these studies have been limited to other viruses and bat species rather than EBOV and fruit bats or insectivorous bats. Therefore, it is very difficult to conclude whether the antiviral responses observed in cells derived from bat species such as black flying fox (*Pteropus alecto*) can be applied to fruit and other insectivorous bats. It will be valuable to carry out similar investigations to determine the immune responses against viruses in bat species that have been recognised as potential natural reservoirs of EBOV. For example, it would be interesting to determine whether these bat associated with EBOV encode a tetherin-like protein that restricts virus replication in cells.

14. CONCLUSION

The fight against EVD through the development of potent vaccines and drugs relies on adequate understanding of the structure, function and mechanism of action of EBOV proteins. Current anti-EBOV therapeutics and vaccines are very promising but treatment regimens that include the use of more than one therapeutic or vaccine may be effective. Increasing the spectrum of vaccines to include other proteins such as VP24 and VP35 may improve their effectiveness.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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