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REVIEW ARTICLE

Review on Ebola virus disease and its treatments

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ABSTRACT

Ebola Virus mainly attack on the interferon responsible for the prevention against viral infection due to inhibition of interferon the virus shows it impacts/ effect on body, having different species, show hemorrhagic fever and may results into individual death. Pathogenesis of disease include infection to monocyte, DCs and interferon, some life supportive care must be provided as per symptoms. vaccines & drugs are developing to treat Ebola infection discussed in this review.

KEYWORDS

Ebola Virus, Disease, Favipiravir, Treatment

INTRODUCTION

In addition to chikungunya, dengue, swine flu and Zika viral diseases. Ebola virus is a potential public health threat of pandemic proportion for India. It is so, on account of human to human transmission of Ebola virus via exudates of patients, absence of licensed vaccine for protection against the disease and of therapeutics for the treatment of disease, continued presence of Ebola virus in its reservoir hosts in the endemic areas of EVD, Ebola virus possessing the properties of category - A biothreat pathogen and high fatality rates in its patients. Since 1976 when EVD was first described, there have been at least 26 outbreaks of EVD in the central and estern region of Africa.

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Out of the two recent EVD outbreaks, smaller one in the Democratic Republic of Congo,the larger one in west Africa in a region comprising of Guinea, Liberia and Sierra Leone is still in progression and by July 19,2015 about 11,269 EVD patients died. Travellers and evacuees from outbreaks region in Africa have carried the disease to Mali,Senegal and Nigeria in Africa and to North America and Europe

One infected person and animal can spread the EVD infection in crowded location such that outbreaks thereafter can assume pandemicity. Unless the invasion of EVD is controlled by allround preparedness, EVD in India, if it is some how get introduced, could rapidly become a pandemic. Preventive measures against the emerging Zika virus disease(ZVD) are being developed using those enunciated against the EVD as the model

In view of the EVD outbreaks and ebola virus emerging as a potent bioweapon in recent year the research on various aspect of the EVD has been growing steadily, internationally.

In the present article, some of the important information about Ebola virus and EVD described in the current scientific literature has been summerised and discussed to serve as introduction on the subject, with the hope that it will spur the greater interest and some research activity on EVD rapid diagnosis, therapeutics and vaccines and other logistics of Ebola Virus Characteristics.

WHAT IS EBOLA?

Ebola virus disease (EVD) is a severe, often fatal illness in humans. EVD outbreaks have a case fatality rate of up to 90%. Ebola first appeared in 1976 in two simultaneous outbreaks, in Nzara, Sudan, and in Yambuku, Democratic Republic of Congo. The latter was in a village situated near the Ebola River, from which the disease takes its name. It has not been reported in humans in the Asia Pacific region as of 31 July 2012. However, with global travel, it is possible that outbreaks in Africa could result in the spread of the virus to Asia.

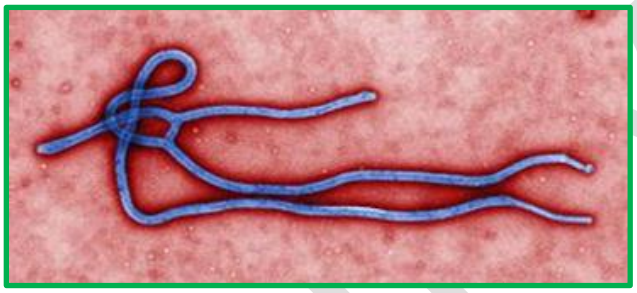


Fig : 1 Ebola Virus

AGENTS

Ebolavirus belongs to the Filoviridae family (filovirus). Ebolavirus comprises 5 distinct species:

Bundibugyo ebolavirus (BDBV)

Zaire ebolavirus (EBOV)

Sudan ebolavirus (SUDV)

Reston ebolavirus (RESTV)

Taï Forest (formerly Côte d'Ivoire ebolavirus) ebolavirus (TAFV)

Four of the five subtypes occur in an animal host native to Africa. BDBV, EBOV, and SUDV have been associated with large EVD outbreaks in Africa, whereas RESTV and TAFV have not. Pathogenicity varies among Ebola viruses, from EBOV, which is highly lethal in humans, to RESTV, which causes disease in pigs and macaques but asymptomatically infects humans.[1][3]

EBOLA VIRUS STRUCTURE

The Ebolavirus genome is a 19 kb single stranded RNA molecule of negative polarity. The seven genes encoded in it lie in the order 3'-Leader-NP-VP35VP40-GP/sGP-VP30-VP24-L-Trailer-5', as shown in Figure. The gene functions are summarized in the Table 1. GP1,2, VP24, VP40, VP30, VP35, NP and L are not only virion structural proteins but also possess a variety of enzymatic and/or regulatory properties for the processes of virion attachment on host cells, virion entry into host cell cytoplasm and viral multiplication (Fig. 3), arrest of antiviral host cell responses, host cell apoptosis and other pathophysiological changes in the host to build a vast reservoir of virus particles for the spread of infection among susceptible animals and humans.

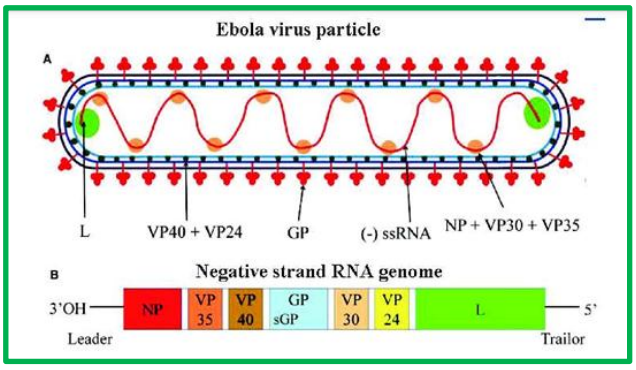


FIG : 2 Ebola Virus Particle

The cis sequences contained in Leader and Trailer regions are essential signals for the control of gene transcription, genome replication and packaging of replicated RNA into virus particles. Each of the seven genes has its own transcription initiation and termination signals flanking it. The open reading frame of each gene, containing its coding region, is flanked by non-translated sequences of

unknown function. Ebola genome transcription occurs in the host cell cytoplasm after the nucleocapsid of the virion has partially uncoated. In the nucleocapsid, RNA genome is associated with the products of NP, L, VP35 and VP30 genes forming a ribonucleoprotein (RNP) complex. In the RNP, expression begins when the L gene product RNA dependent RNA polymerase (RDRP) transcribes the Leader into a 5'-triphosphate Leader RNA and stops. RDRP restarts at the transcription start signal of NP gene. The initiated NP mRNA is capped. At the NP gene transcription termination site, before NP mRNA is released, the RDRP stutters at a stretch of Us and produces a polyadenylated tail on NP mRNA. Then RDRP moves on to transcribe the gene VP35.

Seven genes are transcribed sequentially in the order of their arrangement on Ebola genome. Interestingly, the GP gene transcription results in production of mRNA for three different gene products, namely Pre-sGP (small GP), Pre-GP and Pre-ssGP (small secretary GP), which post-translation get respectively processed into sGP, GP1 and GP2, and ssGP. Normal transcription (bulk) produces mRNA for Pre-sGP, which is the GP gene's primary product (> 70%). Editing in transcription (editing = RDRP reads a template base more than once causing a base addition in the mRNA product) produces PreGP (< 25%) and ssGP (minor , ~5%) mRNAs. At the editing prone site, insertion of an additional A residue at the RNA editing site results in mRNA for Pre-GP protein. Likewise, insertion of two A residues produces ssGP mRNA.

Sr. No.	Gene Name		Protein Function
1	L	RNA DIRECTED RNA POLYMERASE L	GENE/GENOME TRANSCRIPTION AND GENOME REPLICATION AND FORMATION OF NUCLEOCAPSID STRUCTURE
2	GP	ENVELOP GLYCOPROTEIN(A) SMALL SOLUBLE GLYCOPROTEIN(SGP) SYNTHESIZED FROM A SEGMENT OF GP GENE , IS ESSENTIAL (B) GP UNDERGOES PROTEOLYTIC CLEAVAGE TO PRODUCE GP1 AND GP2 WHICH BIND TO EACH OTHER, UNDERGO GLYCOSYLATION AND ACYLATION AND THEIR TRIMMERS GETS INSERTED IN VIRAL MEMBRANE ENVELOPE	PROTECTS GP BY NEUTRALIZING THE HOST ANTI-GP ANTIBODIES; ACTS AS AN ANTI-INFLAMMATORY FACTOR; ITS DELTA PEPTIDE HAS VIROPORIN PROPERTY ESSENTIAL FOR THE ATTACHMENT OF VIRUS TO HOST CELL MEMBRANE AND INTERNALIZATION OF NUCLEOCAPSID OF VIRUS INTO HOST CELL CYTOPLASM; GIVES FILAMENTOUS MORPHOLOGY TO VIRION IN COOPERATION WITH VP40; PROVES TOXIC AND DOWN REGULATES HOST
3	NP	NUCLEOPROTEIN NP	ESSENTIAL FOR RNA ENCAPSULATION; NP IS CHAPERONED BY VP35 TO COIL AND FORM A SHELL ARROUND RNA AGAINST HOST'S IMMUNE RESPONSE

Sr. No.	Gene Name		Protein Function
4	VP24	MEMBRANE ASSOCIATED PROTEIN VP24	ANTI-VIRAL INHIBITOR WHICH IMPAIRS TYPE 1 INTERFERON (IFN)-A/B AND – Γ SIGNALLING; HAS A ROLE IN VIRUS ASSEMBLY AND BUDDING AND IN TRANSCRIPTION AND REPLICATION BY BEING A PART OF NUCLEOCAPSID STRUCTURE A VIRULENCE FACTOR THAT PLAYS ROLE IN HOST ADAPTATION
5	VP30	MINOR NUCLEOPROTEIN (POLYMERASE MATRIX PROTEIN) VP30	TRANSCRIPTION ANTITERMINATOR; SUPPRESSION OF VIRAL RNA SILENCING
6	VP35	POLYMERASE COFACTOR(POLYMERASE MATRIX PROTEIN) VP35	INHIBITS IFN REGULATORY FACTORS 3 AND 7 AND THEREBY BLOCKS IFN-A/B GENE EXPRESSION; PREVENTS ANTI- VIRAL RESPONSE; IMPEDES NEGATIVE CONTROL OF DSRNA DEPENDENT KINASE ON VIRAL REPLICATION; SUPPRESSES VIRAL RNA SILENCING; IS A PART OF VIRION CORE; BINDS TO NP TO UNCOAT RNA GENOME FROM VIRION TO FACILITATE TRANSCRIPTIONAL EXPRESSION AND REPLICATION AND DIRECTS NEWLY SYNTHESIZED NP TOPROGENY RNAS
7	VP40	MATRIX PROTEIN VP40	REQUIRED FOR BUDDING OF VIRUS OUT OF HOST CELL MEMBRANE, LINKS NUCLEOCAPSID AND SURROUNDING MEMBRANE AND GIVES FILAMENTOUS SHAPE TO VIRUS TOGETHER WITH GP AND HELPS TO MAINTAIN STRUCTURAL INTEGRITY OF THE VIRION

LIFE CYCLE

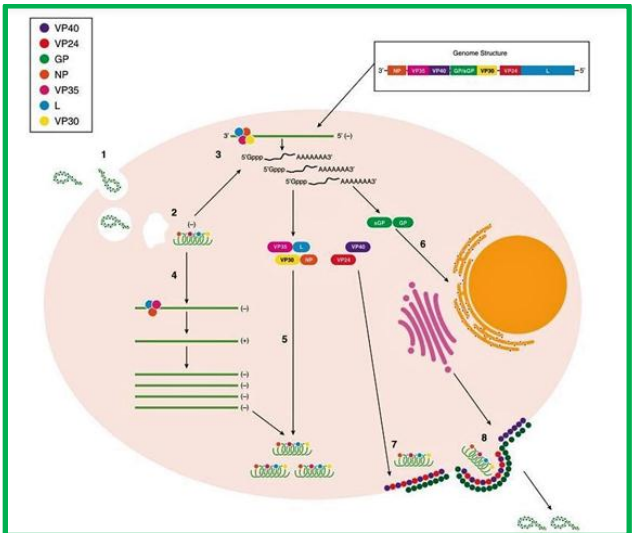


FIG : 3 LIFE CYCLE OF EBOLA VIRUS

Host receptors used by the virus to gain entry include the asialoglycoprotein receptor on hepatocytes; the folate receptor a on epithelial cells; C-type lectins, such as DC-specific intercellular adhesion molecule-3-grabbing nonintegrin and its receptor (on DCs, macrophages, and endothelial cells); and human macrophage lectin specific for galactose/N-acetylgalactosamine (on macrophages)

Once GP binds its receptor, virions enter the cell by process of endocytosis. The acidification of endocytic vesicles is followed by a fusion of virus and host membranes and the release of EBOV into the cytoplasm (Fig. 3).

Endosomal protein NPC1 is an additional entry receptor that has recently been shown to bind EBOV GP through domain C, resulting in a conformational change in GP that triggers membrane fusion. EBOV mRNA synthesis is detectable at 6–7 hr. post infection. The EBOV RNA-dependent RNA polymerase binds a site within the leader region of each negative sense genome and slides along the RNA template, transcribing individual genes sequentially in a 39 to 59 direction.

Each gene is delineated by highly conserved transcription start and stop signals with polyadenylation sites marking the termini of the mRNAs. Additionally, EBOV mRNAs are shown to be capped at the 59 end NP is the most transcribed gene, whereas L is the least transcribed in Vero E6 cells. EBOV transcription is dependent on the presence of transcription factor VP30. The initial transcription and translation of virus genes leads to a buildup of VPs, especially NP, which triggers viral replication.

During replication, the promoter at the 39 end of the genomic RNA drives synthesis of full-length, positive sense antigenomic RNA, which, in turn, serves as a template for production of progeny negative-sense genomes.

When sufficient levels of negative-sense genomes and VPs are reached, they are assembled at the plasma membrane, where VP40 induces budding of filoviruses (Fig. 3).

(1) EBOV gains cell entry by receptor-mediated endocytosis.

(2) Acidification of the endocytic vesicle, followed by fusion of the virus and host membranes, releases the EBOV nucleocapsid into the cytoplasm.

(3) The RNA-dependent RNA polymerase transcribes individual mRNA from the negative-sense genome in a 39 to 59 direction. Each mRNA is capped at the 59 end and contains a poly-A tail.

(4) During replication, the promoter at the 39 end of the genomic RNA drives synthesis of the full-length, positive-sense, antigenomic RNA, which, in turn, serves as a template for the production of progeny, negative-sense genomes.

(5) Nucleocapsid proteins (VP35, L, VP30, and NP) associate with negative-sense genome progeny, whereas

(6) GP and sGP are further modified in the endoplasmic reticulum and Golgi body.

(7) When sufficient levels of the negative-sense genomes and viral proteins are reached, they are assembled at the plasma membrane with membrane-associated proteins (matrix proteins VP24 and VP40 and GP).

(8) Complete virions bud from the cell surface[4]

Transmission

In some past Ebola outbreaks, primates were also affected by Ebola and multiple spillover events occurred when people touched or ate infected primates. When an infection does occur in humans, the virus can be spread in several ways. Ebola is spread through direct contact (through broken skin or mucous membranes in,
e.g., the eyes, nose, or mouth) by the following:

- Blood or bodily fluids (including but not limited to urine, saliva, sweat, feces, vomit, breast milk, and
- semen) of a person who is sick with Ebola;

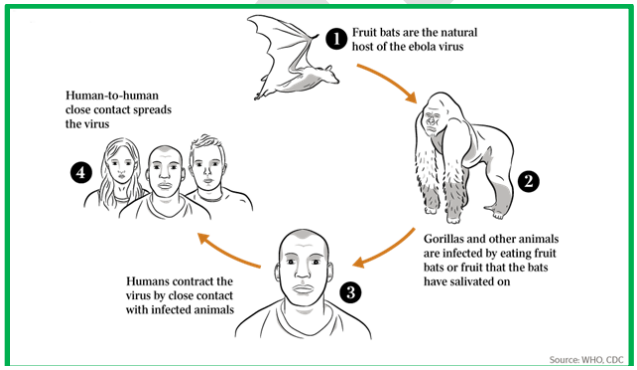


FIG : 4 TRANSMISSION OF EBOLA VIRUS

- Objects (like needles and syringes) that have been
- contaminated with the virus; or
- Infected fruit bats or primates (apes and monkeys).

Ebola is not spread through the air or by water, or in general, by food. However, in Africa, Ebola may be spread as a result of handling bushmeat (wild animals hunted for food) and contact with infected bats. There is no evidence that mosquitos or other insects can transmit Ebola virus. Only a few species of mammals (e.g., humans, bats, monkeys, and apes) have shown the ability to become infected with and spread Ebola virus[2]

PATHOGENESIS

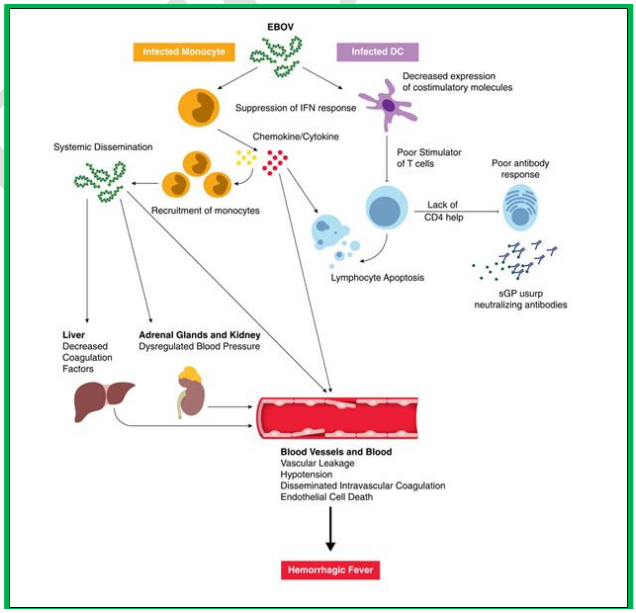


FIG : 5 EBOLA VIRUS PATHOGENESIS

Ebola virus initially and preferentially infects monocytes, macrophages, and DCs. Infection of DCs impairs their maturation and suppresses type I IFN responses, thereby preventing T cell activation. Infection of monocytes and macrophages leads to the robust expression of inflammatory mediators. Secreted chemokines can recruit more monocytes, which act as new targets for viral infection.

Inflammatory mediators, reactive oxygen species, and nitric oxide can induce apoptosis leading to lymphocyte death. The lack of lymphocytes, such as CD4 T cells, inhibits the ability of the virus to induce an Ab response.

Production of EBOV secreted glycoprotein (sGP) usurps any GP-specific Abs that are made. Eventually, the inflammatory cytokines are responsible for vascular leakage. EBOV systemically disseminates to liver, kidneys, adrenal glands, and endothelial cells, which contributes to symptoms associated with haemorrhagic fever.[4]

INNATE IMMUNE RESPONSE AND ITS BLOCKAGE BY EBOLA VIRUS

Interferons are the potentiators of innate immune response. In all types of Ebola infected cells, including macrophages, monocytes and dendritic cells where infection initiates, presence of virus is sensed by the immune system receptors. Presence of Ebola activates both the cytoplasmic receptors, such as; Retinoic acid inducible (RIG)-1 and Melanoma differentiation associated (MDA)-5 and extracytoplasmic receptors examplified by Toll-like receptor (TLR)-3, 7, 8 and 9, altogether termed as RIG-1 like receptors (RLRs). In this cascade, RLRs activate kinases-Tank binding kinase (TBK)-1 and Ikappa- B kinase epsilon (IKKE). Next, TBK-1 and IKKE phosphorylate Interferon regulatory factor (IRF)-3 and IRF-7. Thereafter, IFRs dimerize, transport to nucleus and induce transcription of Type 1 interferons (IFN). Secreted type 1 IFNs then bind to IFN alpha receptor (IFNAR)-1 and IFNAR-2 subunits of IFN receptor. This triggers the Janus kinase (JAK) and Signal transducer and activator of transcription (STAT) signaling cascade. Auto-phosphorylated JAK1 and Tyrosine kinase (TYK)-3 phosphorylate STAT1 and STAT-2. STAT-1 homodimers and STAT-1/ STAT-2 heterodimers get transported to nucleus. Nuclear transport of STAT is enabled by interaction with Karyopherin (KPN)-alpha (nuclear transporters). STATs induce the transcription of IFN stimulated genes (ISG). Among the IGS products are included Interferon inducible transmembrane (IFITM) protein and Tethrin, which respectively block entry of Ebola virion into host cell cytoplasm via interaction with NCP-1 and stop Ebola virion budding by interaction with host cell plasma membrane. The other ISGs, that allow establishment of anti-Ebola state, synthesized are double stranded RNA dependent Protein kinase (PK)-R (PKR), its oligo adenylate synthetase (OAs), RNAse L, RNA specific adenosine deaminase, and Major histocompatibilty (MHC) class 1 and 2 proteins.

Ebola virus deploys its proteins VP35, VP24 and GP1,2 to blunt the IFN mediated innate immune response in multiple ways . VP35 blocks IFN production by suppressing RLR activation, inhibition of IRF-3 phosphorylation and sumoylation of IRF-7 VP24 blocks ISGs expression by blocking nuclear transport of STAT-1 . GP2 of GP1, 2 mislocalizestethrin in plasma membrane such that tethrin cannot interfere with the VP40 based release of Ebola virions[3]

DYSREGULATED ADAPTIVE IMMUNE RESPONSE AFTER EBOV INFECTION

Severe lymphopenia and destruction of lymphoid tissue is one of the hallmarks of Ebola infection (Fig. 6). Loss of peripheral blood CD4+ and CD8+ T cells as well as NK cells has been documented in mice, cynomolgus macaques, and human PBMCs cultures after ZEBOV infection. Loss of B cells is more controversial, with some studies demonstrating apoptosis of B lymphocytes using double-staining for CD20 and TUNEL in mice and

macaques, whereas other studies show no changes in B lymphocyte counts in cynomolgus macaques. In vivo and in vitro studies using TUNEL staining and transmission electron microscopy confirm apoptosis as the main mechanism of lymphocyte loss during ZEBOV infection. In humans, percentages of peripheral blood CD4+ and CD8+ T cells expressing the apoptotic marker CD95 were greater in fatally infected patients compared with healthy individuals. Increased expression of CD95 was also observed in cynomolgus macaques. Increased levels of soluble Fas and 41/7 nuclear matrix protein, which is cleaved and solubilized during apoptosis, have been detected in plasma of patients during the last 5 days of life after infection with ZEBOV. Similarly, an up-regulation of Fas and Fas-ligand mRNA levels was detected using RT-PCR in PBMCs of infected patients. Moreover, mice deficientin the expression of Fas-associated death domain or overexpressing the antiapoptotic molecule BCL2 were resistant to MA-EBOV–induced lymphocyte apoptosis, suggesting lymphocyte death can occur via both extrinsic (death receptor) and intrinsic (mitochondrial) pathways. Furthermore, although patients who survived ZEBOV infection showed an up-regulation of BCL2 mRNA in PBMCs, those who succumbed showed a significant decrease of BCL2 mRNA expression during the terminal stage of infection. The decreased expression of BCL2 was coincident with the loss of CD3, CD8, and T cell receptor b chain variable region mRNA in PBMCs. However, infection of human PBMCs in vitro with ZEBOV did not result in increased expression of Fas but rather an increase in mRNA levels of TRAIL in CD4+ and CD8+ T cells at 7 days post infection. These data suggest that additional inflammatory mediators released in vivo during infection result in the increased Fas expression and that apoptosis of lymphocytes during ZEBOV infection is not due to viral replication (Fig. 6). Indeed, analysis of ZEBOV-infected NHP tissues clearly showed the presence of EBOV antigens within the mononuclear phagocytic cells but not in the lymphocytes, even after in vitro infection.

Some inflammatory mediators produced after ZEBOV infection, such as TNF-a, nitric oxide, and reactive oxygen species, can induce apoptosis. Moreover, 90% of ZEBOV-infected, adherent human macrophages were positive for TRAIL by immunohistochemistry, flow cytometry, and RNA analysis and can, therefore, induce apoptosis in lymphocytes via the extrinsic pathway. The lymphopenia observed during an EBOV infection in part explains the lack of EBOV-specific T and B cell responses. The lack of T cell response is evident by the absence of T cell–derived cytokines (IL-2, IL-3, IL-4, IL-5, IL-9, IL-13) in the plasma of fatally infected patients. In addition, lack of activated T lymphocytes was reported in the peripheral blood of ZEBOV-infected macaques and mice. Because CD4+ T cells are required for B cell isotype class switching, the loss of CD4+ T cells may explain the lack of Ebola-specific IgM and IgG Abs observed in fatally infected patients. Moreover, the EBOV envelope is covered in a dense concentration of N- and O-linked glycans, which interfere with binding of neutralizing Abs. Finally, secreted GPs, which account for about 70% of GP mRNA transcripts, act as decoys that usurp the much-needed neutralizing Abs (Fig. 5). Subversion of the adaptive immune response, coupled with inactivation of the innate immune branch, allows EBOV to disseminate systemically. The magnitude of the recent ZEBOV epidemic and the large number of survivors has provided a unique opportunity to study host immune responses in patients with EVD who survived infection. Four patients

treated at Emory Hospital with various combinations of the Ab cocktail Zmapp (Mapp Biopharmaceutical, San Diego, CA, USA), an siRNA against ZEBOV, a DNA polymerase inhibitor, and convalescent serum as early as 1 d and as late as 10 d after symptom onset exhibited increased frequencies of activated CD8+ T cells, CD4+ T cells, and plasmablasts 2–3 week after the onset of symptoms. Lymphocyte activation coincided with a decline in viral loads during the second week, which reached its nadir by week 3 after symptom onset. EBOV-specific IgG responses peaked 2–3 week after symptom onset, and the strongest T cell responses were directed against ZEBOV NP. Surprisingly, the number of activated T cells remained elevated up to 30 d after discharge, suggesting potential persistence of ZEBOV antigen. A second study of T cell immune responses in patients with EVD, managed at the Ebola Treatment Centers in Guinea (n = 47 in Gu ´ eck´edou; n = 157 in Coyah), showed that, although there was robust CD4+ and CD8+ T cell activation (measured by coexpression of CD38 and HLA-DR) in all EVD cases, an increase in the coexpression of the negative T cell regulators CTLA-4 and PD-1 significantly correlated with fatalities. These studies indicate that dysregulation of the T cell response, in addition to lymphopenia, contributes to EVD pathology.[4]

VASCULAR PERMEABILITY AND COAGULATION DEFECTS

In addition to inducing apoptosis within lymphocytes, the large release of TNF-a from infected monocytes/macrophages can increase endothelial permeability, resulting in vascular leakage. In vitro studies show that increased endothelial permeability is temporally associated with the release of TNF-a from MARV-infected human monocytes/macrophages.

Similarly, the release of nitric oxide, which is an important effector molecule in the homeostasis of the cardiovascular system, can result in the loss of vascular smooth-muscle tone and hypotension. In addition, ZEBOV infection of macrophages leads to the up-regulation of surface TF as well as the release of membrane microparticles containing TF, resulting in the over activation of the extrinsic pathway of coagulation and the development of disseminated intravascular coagulation.

Expression of TF is further up-regulated by proinflammatory cytokines, notably IL-6, which are abundant during acute ZEBOV infection, exacerbating the intravascular coagulation phenotype.

In addition, ZEBOV-induced paralysis of the host response facilitates viral dissemination to hepatocytes, adrenal cortical cells, and endothelial cells of connective tissue in cynomolgus macaques (Fig. 5).

Hepatocellular necrosis results in decreased synthesis of coagulation proteins, whereas infection and necrosis of adrenocortical cells may negatively affect blood pressure homeostasis, leading to hemorrhage.

Coagulation abnormalities are initiated early during ZEBOV infection in cynomolgus macaques. Specifically, a dramatic decrease in plasma levels of anticoagulant protein C occurs as early as 2 daypostinfection. This is followed by an increase of both tissue plasminogen activator, which is involved in dissolving blood clots, and fibrin-degradation products (D-dimers) at day 5 postinfection.

Thrombocytopenia and prolonged prothrombin time are indicators of dysregulated blood coagulation and fibrinolysis during ZEBOV infection and may manifest as petechiae,

ecchymoses, mucosal hemorrhages, and congestion. Toward the terminal stage of the infection, and after the onset of hemorrhagic abnormalities, ZEBOV replicates in endothelial cells. However, although infection of endothelial cells is thought to have a role in the pathogenesis, the molecular mechanisms of endothelial damage are not yet fully understood.[4]

LONG TERM HEALTH OUTCOMES IN EBOV SURVIVORS

The recent ZEBOV outbreak in West Africa resulted in an unprecedented number of survivors (;17,000) and highlighted the complexity of EVD sequelae in clinically recovered patients (Fig. 6). One retrospective study collected health status, functional limitations, demographics, blood chemistry, hematology, and filovirus Ab titers from 49 survivors and 157 seronegative contacts, 29 mo after the 2007 Bundibugyo outbreak in Uganda. Results showed that, although no differences in blood analysis were observed, survivors were at a significantly greater risk for ocular problems (retro-orbital pain and blurred vision), loss of hearing, difficulty swallowing, difficulty sleeping, arthralgia, abdominal and back pain, fatigue, impotence, severe headaches, memory problems, and confusion. More recently, a study in which clinical and laboratory records of surviving patients treated in Port Loko, Sierra Leone, were assessed showed a higher incidence of arthralgia, ocular symptoms (including uveitis), and auditory problems. Moreover, a higher ZEBOV viral load at clinical presentation was associated with a higher incidence of uveitis and other ocular symptoms. A second study of surviving patients showed increased incidence of anorexia, arthralgia, myalgia, and chest/back pain (Fig. 6). Further investigation of recovering survivors revealed that ZEBOV persists in the semen, ocular fluid, cerebrospinal fluid, placenta, and amniotic fluid. A study of 93 male survivors in Sierra Leone showed that 100% of men (n = 9) who provided a semen specimen 2–3 months after the onset of EVD had positive qRT-PCR results despite absence of viremia. Of 40 samples obtained 4-6 months after onset, 26 tested positive (65%), whereas 11 of 43 specimens (26%) collected at 7–9 months after onset were positive. Recently, genetic sequencing of ZEBOV strain confirmed a female patient acquired Ebola virus via sexual transmission from a survivor whose semen tested positive for ZEBOV by qRT-PCR 199 days after his recovery. These data suggest that infectious virus, not only viral RNA, can persist in the semen for months after viremia ceases. In one case, although a tear-film specimen and peripheral blood tested negative for ZEBOV RNA by qRT-PCR, virus was detected in the aqueous humor 9 week after blood and urine tested negative for ZEBOV by qRT-PCR. ZEBOV was also detected in the cerebral spinal fluid of a nurse who developed meningitis 9 months after recovery from EVD. Finally, ZEBOV persisted in amniotic fluid and placenta after clearance from the blood in 2 pregnant women, resulting in the delivery of stillborn fetuses in both cases. Collectively, these observations indicate that ZEBOV can persist in organs that were traditionally considered immune privileged sites (Fig. 6). The ability of immune cells to access these sites (anterior chamber of the eye, central nervous system, testes, and pregnant uterus) is limited to reduce the risk of irreparable damage to these critical organ system.

The persistence of ZEBOV in these sites well after recovery raises many questions regarding the mechanisms and the kinetics by which this virus gains access to, and is able to persist in, these sites.

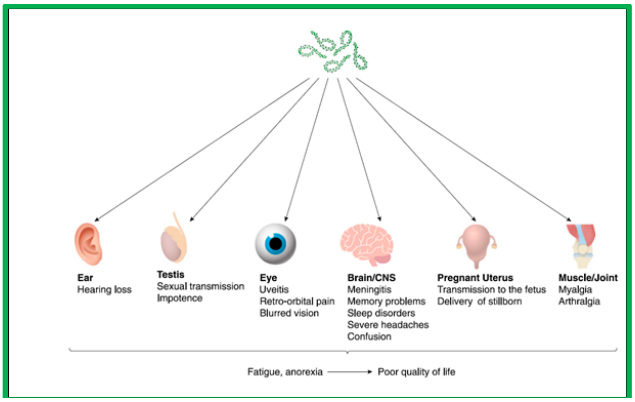


FIG. 6 LONG-TERM POST-EBOV CONSEQUENCES

HOST ALLELES THAT PROTECT AGAINST EBOLA DISEASE

In the course of Ebola outbreaks in Central and Western Africa, a general observation is that people responded to Ebola differentially. Whereas many developed severe symptoms and died, some showed symptoms of the disease but survived and a third group resisted the disease completely This indicated that there may be some human gene/s allele(s) that made people resistant to Ebola infection/disease. Mutant forms of human genes HLAB (Human Leukocyte Antigen-B) called Bx07 and Bx14 have been observed to render their bearers survive the Ebola infection. Contrari-wise the alleles Bx67 and Bx15 make their bearers susceptible to Ebola infection leading to their death. It has been shown that the NP and VP35 proteins of the virus bind tightly to Bx07 gene product as compared to Bx67 protein. Thus, there may be depletion of the functional NP and VP35 proteins in Bx07 Bx07 persons.

Skin cells from Niemann-Pick C1 (NPC1) gene mutant human homozygotes were observed to be resistant to in vitro Ebola virus infection. NPC1 non-function causes in the mutants a neurodegenerative disorder due to defect in cholesterol transport and related functions. It is known that mitochondria have intimate interaction with nuclear encoded NPC1.

This indicates that there could be human mitochondrial haplotypes who may tolerate Ebola infection. Mice mutated in their NPC1 gene have been observed to resist Ebola infection.

A study related with response of Ebola infection in recombinant inbred lines of mice revealed that mice lines were of three types, those that resisted Ebola, another group that were susceptible to Ebola leading to death in mice and a group in which Ebola infection was largely asymptomatic. Among the resistant mice, alleles of TIE1 and TEK genes were present such that the mice bearing them had a relatively more active Coagulation pathway due to better regulation of fluid passage in blood vessels.

Discovery of Ebola resisting genes and mechanism of their action will be greatly valuable in the design of vaccines and discovery of new therapeutics. For example, an inhibitor of NPC1 has been discovered.[3]

DIAGNOSIS

Clinical diagnosis of Ebola infection is safely and most reliably accomplished by the use of ready-made kits of standardized reverse transcriptase polymerase chain reaction (RT-PCR) , which detects the presence Ebola RNA in blood or plasma and by using enzyme linked immunosorbent assay (ELISA), which detects specific viral protein/antigen in serum, plasma or whole blood (Table 2). Biosafety level 4 conditions are used while performing the tests. The tests are performed on persons who have a history of presence among EVD patients or in the area of outbreak in preceeding 23 days, within 0-2 days of the appearance of initial symptoms, especially rise in body temperature.

False positive or negative results are avoided by conduct of ELISA or other test(s) and RT-PCR tests on the suspected patients of EVD. RT-PCR of urine and breast milk of lactating mothers and semen of men recovering from Ebola infection has been recommended . New automated RT-PCR blood tests, quick and easy to perform have proved accurate in field applications. A 37 minutes test detects Ebola RNA load from finger prick in diseased and semen, breast milk and eye fluids in post-recovery patients.

Recently, three rapid point-of-care tests have been described, which complement the RT-PCR test (Table 2). The dipstick immunoassay is performed by placing a finger-pricked drop of blood on a paper strip which detects in the blood presence of VP40 matrix protein of Ebola virus as a band. The test has been shown to be 100% sensitive and 92% specific, using RT-PCR test as the standard. The kit needs to bemaintained at 4oC, otherwise no other external instrumentation is necessary. Another strip-test involves magnetic nanoparticlebasedimmunochromatography. The reaction of antibody against glyoprotein GP of Ebolavirus, that is coated on the paper probe, with antigen present in the blood sample is visualized as colour change on the strip. A colour coded paper strip diagnosis based on multiplex lateral flow technology simultaneously detects Ebola, Yellow fever and Dengue virus infection(s) in persons having fever (initial symptoms of Ebola and other diseases). In this test, yet to be commercialized, antibodies to viruses are conjugated to silver nanoparticles of different sizes and electrostatically absorbed to paper in different regions of paper strip. Upon application of test serum, virus-wise antigen-antibody (NSI protein of Yellow fever and Dengue viruses and GP of Ebola virus) interaction(s) allow development of red colour for Ebola, orange for Yellow fever and green for Dengue, in their respective areas on paper strip. This 10 min test will cost as much as a pregnancy test (~Rs. 150), after which it is designed (Yen et al., 2015). have reported an optofluidic analysis system that detects presence of 0.2 plaque forming units/mL in finger pricked blood sample in under ten minutes at point of care. This test is as sensitive as the RT-PCR, the Gold standard test for EVD

TABLE 2. RELIABLE , SIMPLE & FAST DIAGNOSTIC TEST FOR EBOLA VIRUS

Attribute	Quantitative real time reverse transcription polymerase chain reaction (qRT-PCR)a,b	Antigen-capture enzyme linked immunosorbent assay(ELISA)	Dipstick immuno-assay Antigen Rapid test	Magnetic nanoparticle-based immunochromatographic strip (Nanozyme-strip)e	Multiplexed lateral flow diagnostic

Principle	Amplification of specific genome segment of ebola virus by the RNA present in same sample	Detection of ebola specific antigen in the sample	As in ELISA	Anti-Ebola virus (EBOV) antibody coated probe recognizes,seperates and visualizes EBOV on strip	Simultaneous detection of Ebola , yellow fever and dengue viruses
Sample from the presumably infected human	Blood or Plasma	Serum Plasma or Whole blood	Plasma or whole blood latter by finger prick	As in ELISA	Blood serum
Sterilisation of the sample	Treatment with a chaotrope such as trizol or guanidiumisothiocynate	Exposure to high temperature or gamma radition			
Ebola gene/protein whose presence is tested	Nucleoprotein (NP) gene domain conserved in Zaire & Sudan species of Ebola virus	NP epitope defined by a sequence of 26 aminoacid near the c terminus of NP protein	VP40 matrix proteins	Glycoprotein (GP)	NS1 protein of Yellow fever and Dengue viruses and GP of Ebola viruses
Whether the readymade kit(s) is/are available	Newsana diagnostic test for Ebola produced by Primer Designed Ltd (a 90 min assay) test developed by the Department of Defense , US Government; AgPath-ID One Step RT-PCR of Applied Biosystems; and EBOV Accu Power Real Time PCR kit of Bioneer	VEGA Ebola Test Device produced by Vega Medicine Ltd; Anti Zaire-Ebola Virus Nucleoprotein (NP) IgM ELISA kit of Alpha Diagnostic International; eZYSCREEN produced by Vedalabs and Atomic Energy Commission (France) (a 15 minutes assay)	CorgenixInc Broomfield Co. Colorado, USA (approved by World Health organisation (a 15 minutes test costs about $ 15 or Rs 1000)	A patent has been applied for and the test is being used in the field in West Africa under the auspices of Center for Disease Control (CDC)	This 15 minute test requires U.S. Food and Drug Administration approval for its commercial production

STERILIZATION OF MATERIALS CONTAMINATED WITH EBOLA VIRUS

Ebolavirus retains its infectivity at room temperature for several days. To stop spread of Ebola infection, it is impotant to sterilize surfaces and materials contaminated with Ebola (Ebola containing exudates from EVD patients). Ultra violet light (UV) inactivates Ebola virions. UV-emitting tubes, lamps and torches are available to UV-light even the whole rooms to sterilize Ebola containing surfaces. Gamma rays (1.2 x 106 rads) also inactivate Ebola virions present in liquid or solid materials; Gamma cells are available to irradiate materials. Boiling of materials for five minutes or exposing of materials to 60 degree Celsius for 30 min are also effective in inactivating Ebola. Diluted ethyl/methyl alcohol (10%), acetic acid (3%), chlorine solution (5%), Sodium hypochlorite (5.25%) and gluteraldehyde (1%) are readily available Ebola disinfectants. Ether, Sodium deoxycholate, βpropiolactones, 1,5-iodonapthylazide, guanidiumisothyocynate also render Ebola virions in-infective [5][6]

MEASURES TO COUNTER EBOLA VIRUS DISEASE

The reservoirs of Ebola virus variants, persistent in wild animal hosts, in areas of their endemicity, and acts of bioterrorism can be the cause of outbreaks of EVD in future. Secondary infections from Ebola infected foreign visitors/travellers could make such outbreaks pandemic. Counter measures against EVD

are urgently required to stem the ongoing outbreak in West Africa and any future outbreak(s) within or outside of the region of common existence of Ebolavirus. EVD would be best controlled by a single dose vaccination not requiring a booster dose and providing protection for as long as ten years. The other course is to counter EVD with antiviral therapeutics. Both vaccines and therapeutics are desired that can ward against Ebola infection and/or cure the EVD. Intensive efforts have been in progress in both the directions.[3]

ANTI-EBOLA VACCINES

The general idea underlying the ongoing anti-Ebola vaccine development programme is to overexpress, in the vaccinated persons, Ebola proteins such as GP, VP40 and NP that are known to themselves cause no serious disease. GP is present on the surface of the virus and is highly immunogenic, but evolves faster than other genes. The GP gene, GP and VP40 or GP and NP genes are recombinationally inserted into the genomes of viruses that do not cause serious disease or produce no or only minor side effects. GP, VP40 and NP proteins synthesized from Ebola vaccine virus genomes serve as small antigens to incite effective and B and T cell mediated anti-Ebola immunity. An Ebola virus whole genome (minus VP30) vaccine to prime host's immune system against many proteins of virus is also under testing. It is desired that the design of vaccines should be such that they can be produced readily in large quantities.There are several anti-Ebola vaccines under development. A few of these mentioned below have reached advanced clinical testing on human volunteers, following their effectivity tests on non-human primates.

VSV ΔG/EBOV GP or rVSV-ZEBOV vaccine:
Presently, this is the most promising anti-Ebola vaccine. The Zaire Ebola GP gene is added on to the replication competent genome of Vesicular Stomatitis Virus (VSV, a rabies family zoonotic virus infective on insects, cattle, horse and pigs causing flu-like disease in humans) incapacitated for disease causation by deletion of its own glycoprotein gene. Non-human primates, both native and previously

VSV vaccinated, were completely protected by intramuscular VSV ΔG/EBOV GP vaccination, against challenge of lethal dose of EBOV four weeks after vaccination. Post vaccination, animals did not develop fever or any other adverse effect. It proved safe and effective on human volunteers and phase III trial is in progress. Recent final analysis of a ring vaccination in Guinea (West Africa) has indicated that rVSV-ZEBOV is effective and safe in preventing EVD at population level. Delivered via ring vaccination strategy, the vaccine is able to control EVD outbreak.

Ad-CAG opt ZGP and HPIV3/EboGP: These vaccines are another promising first line defence against EVD. In the Ad-CAG opt ZGP the vector is replication incompetent Adenovirus serotype 5 genome into which is inserted the ZEBOV GP gene optimized for over-expression in human cells. In HPIV3/EboGP, the vector for GP gene is human parainfluenza virus type 3. A single nasal spray of formulated either vaccine gave long lasting protection to non-human primates. The vaccine induced strong response in CD8+ and CD4+ T cells and Ebola GP specific antibodies in mucosa as well as systemically. When available for human use, this type of non-injectable vaccines will have greater affordability due to simplification of transport, storage and administration of vaccine.

Ch-Ad3-EBOZ vaccine: Several versions of this vaccine are under development; they all have replication incompetent Chimpanzee Adenovirus type 3 genome as the vector, but vary in composition of the GP gene insert: GP of Zaire Ebola, GPs of Zaire and Sudan Ebola or GP of Marburg virus. These are used monovalently as well as bivalently (Zaire + Sudan and Marburg GP vaccines together). Intramuscular administration of a dose of Ch-Ad3-EBOZ followed by a booster dose of pox virus-GP vaccine eight weeks later gave full protection, as observed at 10 months after initial vaccination, in non-human primates.

VP30 minus whole genome vaccine: This vaccine uses whole Ebola virus from which VP30 gene has been deleted. The virus particles are inactivated with hydrogen peroxide. One dose of the vaccine protected cyanomolgous monkeys against the fatal dose of Ebola virus.

More than one kind of vaccine/s referred to here that are undergoing clinical trials in Africa are expected to be used and commercialized in the period of ongoing West Africa outbreak.

To control EVD outbreaks due to zoonotic transmission of Ebola virus to humans, development of animal species-specific anti-Ebola selfdisseminating vaccine(s) is in progress. Animal species specific cytomegaloviruses, that have little or no effect on human health, are being altered to carry Ebola genes such as GP. Such vaccines are expected to immunize animals in the wild against Ebola virus infection.[3]

THERAPEUTICS AGAINST EBOV

Strategies for developing experimental, postexposure treatments against EBOV focus on

1) preventing the development of filovirus-associated coagulopathies (recombinant nematode anticoagulant protein and recombinant human activated protein C);

2) inhibiting viral replication or translation, such as nucleotide analogs (Favipiravir [Toyama Chemical, Tokyo, Japan], BCX4430 [BioCryst Pharmaceuticals, Durham, NC, USA], Brincidofovir [Chimerix, Durham, NC,

USA]) and antisense therapeutics (PMOs and siRNA); or

3) limiting viremia and virus spread (mAb cocktails). Several of these postexposure therapeutic candidates are currently in clinical trials[4]

Favipiravir (T-705), developed by Toyama Chemical, is an oral nucleotide analog that has been licensed for the treatment of influenza. It inhibits viral RNA-dependent RNA polymerase by directly competing with GTP after it is converted to its active metabolite form (ribofuranosyl triphosphate). Favipiravir has been shown to suppress ZEBOV replication in Vero E6 cells when added 1 h after infection.[7]

The adenosine nucleoside analogue BCX4430 is a direct-acting antiviral drug under investigation for the treatment of serious and life-threatening infections from highly pathogenic viruses, such as the Ebola virus.

Cellular kinases phosphorylate BCX4430 to a triphosphate that mimics ATP; viral RNA polymerases incorporate the drug's monophosphate nucleotide into the growing RNA chain, causing premature chain termination.

BCX4430 is active in vitro against many RNA viral pathogens, including the filoviruses and emerging infectious agents such as MERS-CoV and SARS-CoV. In vivo, BCX4430 is active after intramuscular, intraperitoneal, and oral administration in a variety of experimental infections.[8]

Brincidofovir (CMX001), developed by Chimerix, is a lipid conjugate of cidofovir (known to inhibit replication of DNA viruses, including cytomegalovirus and adenovirus) that inhibits ZEBOV replication in vitro, although the mechanism is unknown [9] Because of the few new EVD cases, a clinical trial study of Brincidofovir has been terminated.[10]

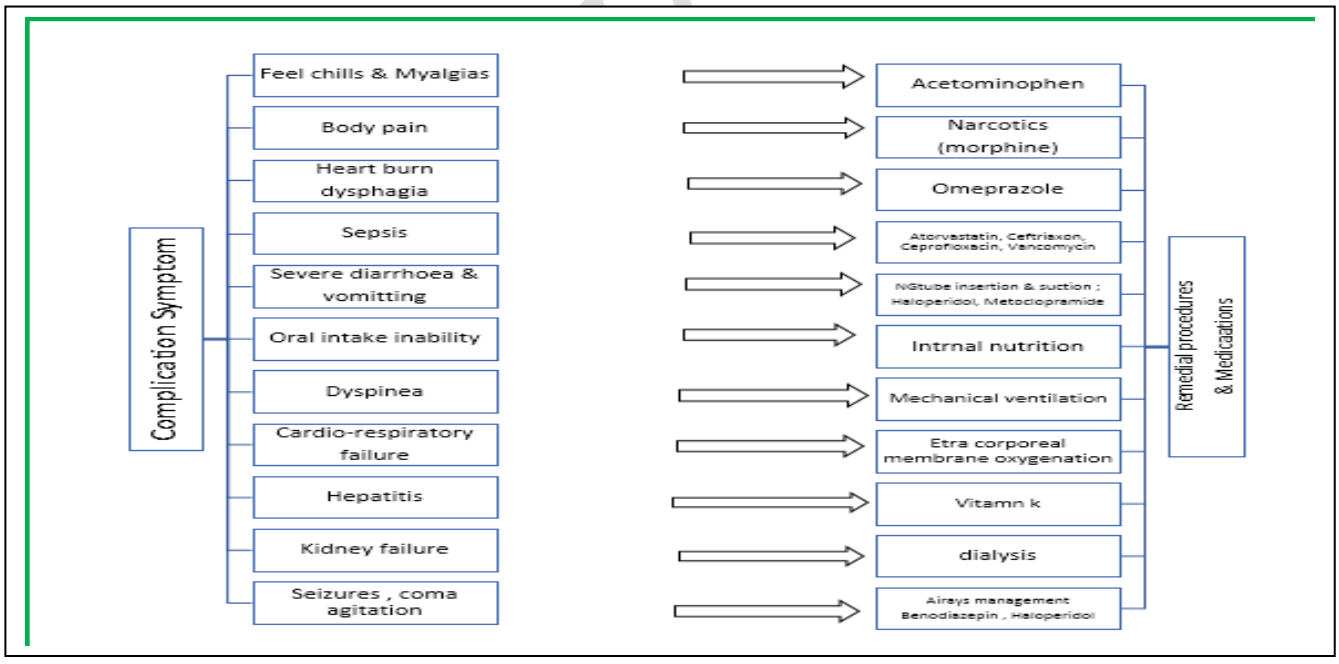


FIG: 7 SUPPORTIVE CARE

LIFE SAVING SUPPORTIVE CARE

Until vaccine and medicine based therapies for EVD become available, supportive medical care, which nurses patients to limit intensity of disease in his/her body, remains the main treatment. The role of experimental therapies (such as Z Mapp, BCX4430, Favipiravir, Brincidofavir and F4-6 etc) is not yet firmly established, although their compassionate use has been in vogue. Fig. 7 gives a profile of the currently available, experience based, supportive care treatments of EVD. Supportive treatment essentially consists of ensuring that patient's body maintains fluid volume, balance of electrolytes, oxygen status, blood pressure and kidney function.

It has been noted that the EVD patients who start to receive supportive care as soon as the post-incubation period, early symptoms appear have better chances of survival than those who receive the medicinal care in late stages of infection.

Recently, Chiappelli et al., (2015) have advised inclusion of Selenium replishment as an element of palliative care. Patients under supportive care take months to recover. Only those are considered as cured in whom virus is found absent from their blood/plasma and other body fluids. The so-called cured/convalescent patients require continued attention for years about one or more of the following medical ailments: psychosis, photophobia, excessive tearing from eyes, sloughing of skin, hairloss, deafness, myelitis, pericardiatis, orchitis, hepatitis and secondary viral, bacterial, fungal or other infections.[3]

STEPS TO AVOID IMPENDING EBOLAVIRUS DISEASE EPIDEMIC IN INDIA

On account of their stability, rapid transmissibilty and fatal disease causing properties, Ebolaviruses have been described as class A bioterror agents. Ebolaviruses are reservoir among the forest animals in Africa. Since it is impossible to eliminate the Ebola reservoir in wild forest animals, EVD outbreaks are expected to occur periodically in different countries of Africa. Eating semi-cooked Ebolavirus containing bush meat which is traded is the main and continuing factor for Ebolavirus outbreaks in Africa (European Centre for Disease Prevention and Control 2014; Casanova and Weaver 2015). Importation of EVD into countries outside of areas of outbreak will remain a lurking danger. Entry and spread of Ebolavirus in densely populated cities of India will be disastrous. Residents of super-denselypopulated shanty/slum colonies in the cities will be especially vulnerable to Ebolavirus infection. Within India, virus infection could spread from its epicentre to other cities by movement of infected persons. The general absence of immunity against Ebolavirus and population dynamics in densely populated areas in India dictate that the country should prepare for any sudden challenge of EVD importation.

A variety of steps, listed below, should be taken urgently and the process improved on a continuous basis. Guidelines prepared by the World Health Organization, Centre for Disease Control (CDC, USA) and National Institute of Allergy and Infectious Diseases (NIAID, USA) are required to be strictly implemented for the safety of travellers and surveillance of travellers by immigration officials and ship and airline services. General public needs to be made aware of the EVD and hygienic control of infection. The topic of infectious diseases, including EVD should be now included in the curricula of school education. Medical and nursing colleges must have practical and theory courses emphasizing on the emerging infectious diseases, including Ebola. Teams of doctors, nurses and epidemiologists must be composed in all the states and union territories and suitably trained about management of EVD. Diagnostic laboratories should be established in all the major cities. Mobile diagnostic laboratories should also be constructed with

desired safety levels for conducting tests, at foci of disease spread. Hospitals should be identified which will function as the EVD treatment centres under whose charge quarantine facilities will become operational on short notice.

Procedures for fool proof case management, contact tracing, quarantining, treatment and safe disposal of contaminated materials and cadavers will be standardized, published and kept ready. The materials to be used in treatment, safeguarding of care givers and prophylaxis will be stockpiled and steps taken to inventorise their speedy procurement and/or manufacture. Some Indian laboratories should initiate research on development of pre- and post-infection drugs effective in preventing and treating EVD, using suitable containment facilities (P4). This work should be broad based against communicable diseases, since there is evidence that some of the therapies may be common to several different virus caused diseases

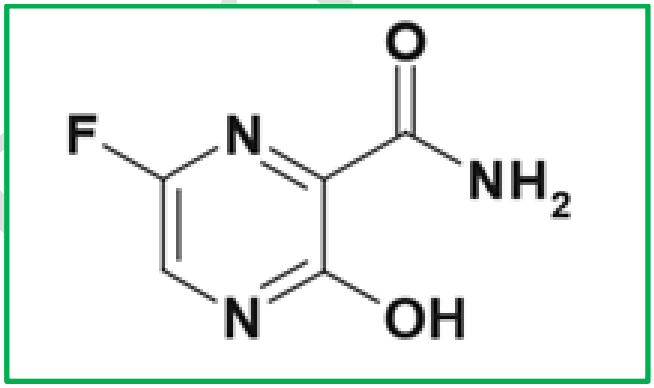
Quarantining of infected people is considered the most effective mode for stopping the spread of Ebola virus disease outbreak. To arrest the spread of disease, areas housing the infected persons will need to be isolated from the rest. The needs of quarantined people will be met as above. The procedure to combat EVD epidemic will have to be updated as the vaccines and therapies already at the advanced stages of clinical trial get approved and begin to get manufactured.[3]

SCOPE OF STUDY

HOW FAVIPIRAVIR WORKS

Existing anti-influenza virus drugs inhibit the virion M2 ion channel (amantadine and rimantadine) or the viral neuraminidase (oseltamivir, zanamivir). The mechanism of action of favipiravir through direct inhibition of viral replication and transcription is therefore unique among anti-influenza drugs. Since RdRP domains are not present in human cells, and are conserved among RNA viruses, this distinct specific mechanism targeting RNA viral polymerases makes favipiravir an attractive drug candidate.

Favipiravir (T-705; 6-fluor-3-hydroxy-2-pyraxinecarboxamide) is a synthetic guanidine nucleoside analogue that has demonstrated potent broad-spectrum antiviral activity in vitro and in vivo against multiple families of RNA viruses



Chemical structure of Favipiravir

Favipiravir is a selective and potent inhibitor of influenza viral RNA polymerase, and effective against all subtypes and strains of influenza viruses including ones sensitive or resistant to marketed neuraminidase and M2 inhibitors. Favipiravir demonstrated anti-viral activities against other RNA viruses. These data clearly suggest that favipiravir is a promising drug for the treatment of infections by not only influenza virus but also a wide range of RNA viruses. On the other hand, favipiravir has a risk for teratogenicity and embryotoxicity. Therefore, the Ministry of Health, Labour and Welfare granted conditional marketing approval with strict regulations for its production and clinical use. In this review, we described the mechanisms of action of favipiravir, a broad spectrum of anti-viral activities in vitro, and

therapeutic potential in animal infection models of a wide range of RNA viruses.

Mechanisms of action of favipiravir Favipiravir inhibited the replication of viral genome, which was the most manifested in the middle of viral proliferation cycle in a time-of-drugaddition test. Anti-viral activity of favipiravir was attenuated in the presence of purine nucleosides or purine bases, indicating competition of favipiravir with purine nucleosides rather than pyrimidine nucleosides. Madin Darby Canine Kidney (MDCK) cells are well used as an in vitro assay of influenza virus. MDCK cells were treated with favipiravir, and cellular metabolites were analyzed by HPLC. Favipiravir ribofuranosyl-5B-triphosphate (favipiravirRTP), favipiravir ribofuranose (favipiravir-R) and favipiravir ribofuranosyl-5B-monophosphate (favipiravir-RMP) were detected. These results suggest that the activation of favipiravir takes place once it is incorporated into cells. Favipiravir-RTP was chemically synthesized and was tested for the inhibition of RNA polymerase activity of influenza virus as assessed by incorporation of 32P-GTP. FavipiravirRTP inhibited the viral RNA polymerase activity in concentrations ranging from nanomolar to micromolar. None of favipiravir

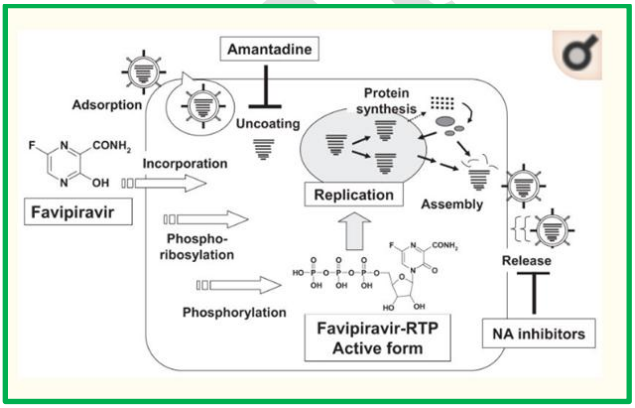


FIG. 8 MECHANISM OF FAVIPIRAVIR

and favipiravir-RMP affected influenza RNA-dependent RNA polymerase (RdRp) at 100μmol/L.

These results indicate that favipiravir exerts its anti-viral activity as a pro-drug, since favipiravir is intra-cellular lyphosphoribosylated to be an active form, favipiravir RTP, which inhibits the viral replication by interacting with viral RNA polymerase. The mechanism of the interaction of favipiravirRTP with RdRp molecule has not been fully elucidated. It is hypothesized that favipiravir may be misincorporated in a nascent viral RNA, or it may act by binding to conserved polymerase domains, thus preventing incorporation of nucleotides for viral RNA replication and transcription.[11]

HOW INTERFERON WORKS DURING VIRAL INFECTION

Interferons were the first family of cytokines to be discovered. In 1957, researchers observed that susceptible animal cells, if they were exposed to a colonizing virus, immediately became resistant to attack by other viruses. Humans produce at least three distinct classes, IFN-α, I FN-β and IFN-γ. Interferons induce a wide range of biological effects. Generally, type I interferons induce similar effects, which are distinct from the effects induced by IFN-γ.

The most pronounced effect of type I interferons relates to their antiviral activity, as well as their anti-proliferative effect on various cell types, including certain tumour cell types. Anti-tumour effects are likely due not only to a direct anti-proliferative effect on the tumour cells themselves, but also due to the ability of type I interferons to increase NK and T-cytotoxic cell activity. These cells can recognize and destroy cancer cells.

The molecular basis by which interferons promote their characteristic effects, in particular antiviral activity, is understood at least in part. Interferon stimulation of the JAK–STAT pathway induces synthesis of at least 30 different gene products, many of which cooperate to inhibit viral replication. These antiviral gene products are generally enzymes, the most important of which are 2'–5'oligoadenylatesynthetase (2,5-An synthetase) and the eIF-2α protein kinase.

These intracellular enzymes remain in an inactive state after their initial induction. They are activated only when the cell comes under viral attack, and their activation can inhibit viral replication in that cell. The 2,5-An synthetase acts in concert with two additional enzymes, i.e. an endoribonuclease and a phosphodiesterase, to promote and regulate the antiviral state (Figure 9).

Several active forms of the synthetase seem to be inducible in human cells; 40 kDa and 46 kDa variants have been identified that differ only in their carboxy terminus ends. They are produced as a result of differential splicing of mRNA transcribed from a single gene found on chromosome 11. A larger 85–100 kDa form of the enzyme has been detected, which may represent a heterodimer composed of the 40 and 46 kDa variants.

The synthetase is activated by double-stranded RNA (dsRNA). Although not normally present in human cells, dsRNA is often associated with commencement of replication of certain viruses. The activated enzyme catalyses the synthesis of oligonucleotides of varying length in which the sole base is adenine (2'–5'An). This oligonucleotide differs from oligonucleotides present naturally in the cell, in that the phosphodiester bonds present are 2'–5' bonds. The level of synthesis and average polymer length of the oligonucleotide products appear to depend upon the exact inducing interferon type, as well as on the growth state of the cell.

The sole biochemical function of 2'–5'An (and hence 2'–5'An synthetase) appears to be as an activator of a dormant endo-RNase, which is expressed constitutively in the cell. This RNase, known as RNase L or RNase F, cleaves all types of single-stranded RNA (ssRNA). This inhibits production of both viral and cellular proteins, thus paralyzing viral replication. Presumably, cellular destruction of the invading ssRNA will be accompanied by destruction of any additional viral components. Removal of dsRNA would facilitate deactivation of the endo-RNase, allowing translation of cellular mRNA to resume. A 2'–5' phosphodiesterase represents a third enzymatic component of this system. It functions as an off switch, as it rapidly degrades the 2'–5'An oligonucleotides. Although this enzyme also appears to be expressed constitutively, interferon binding appears to increase its expression levels in most cells.

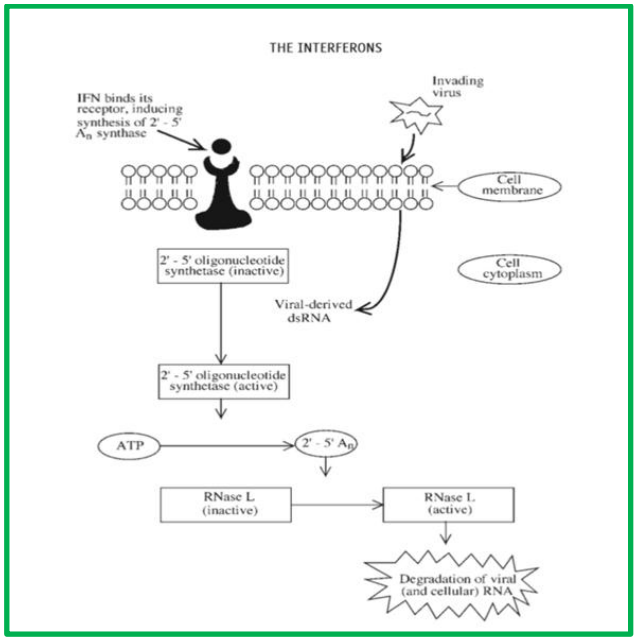


Fig 9. How the 2'–5' synthetase system promotes its antiviral effect

The eIF-2α protein kinase system

Intracellular replication of viral particles depends entirely upon successful intracellular transcription of viral genes with subsequent translation of the viral mRNA. Translation of viral or cellular mRNA is dependent upon ribosome formation. Normally, several constituent molecules interact with each other on the mRNA transcript, forming the smaller ribosomal subunit. Subsequent formation/attachment of the larger subunit facilitates protein synthesis.

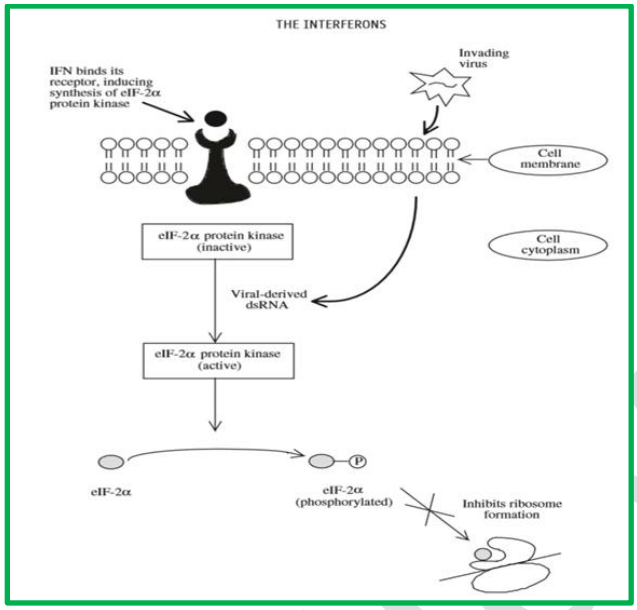


FIG.10. OUTLINE OF HOW THE EIF-2A PROTEIN KINASE SYSTEM PROMOTES AN ANTIVIRAL EFFECT

Exposure of cells to interferon normally results in the induction of a protein kinase termed eIF-2α protein kinase. The enzyme, which is synthesized in a catalytically inactive form, is activated by exposure to dsRNA. The activated kinase then phosplorylates its substrate, i.e. eIF-2α, which is the smallest subunit of initiation factor 2 (eIF2). This, in turn, blocks construction of the smaller ribosomal subunit, thereby preventing translation of all viral (and cellular) mRNA (fig.10)[11]

CONCLUSION

In this review we discuss many things in relation to Ebola virus , the above given mechanism shown by the the interferon inhibited by the Ebola virus which leads to developing ebola virus efficient and the drug which is discuss i.e Favipiravir, The adenosine nucleoside analogue BCX4430 , Brincidofovir (CMX001) , acts against virus by inhibiting viral replication . The supportive care somewhat reduce the symptoms by Medications & anti-ebola vaccines for immunisation. Somewhat if the drugs can be increase the interferon activity can be useful fight aginst ebola.

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