Ebola Virus Immuno-Evasion and Cellular Dysfunctional Mechanics: A Bio-Terrorizing Agent of Zoonotic Origin

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Abstract -: **Ebola virus disease remains one of the most deadly epidemic viral disease in humans, characterized by severe hemorrhagic fever, caused by Ebola viruses – an aggressive viral pathogen of a zoonotic origin. A robust immune response against pathogens requires a coordinated-synergistic activities of both innate and adaptive immunological response. However, Ebola virus attacks immune cells to compromise both innate and adaptive immunological responses against their cytopathic induction by adopting myriads of biochemical mechanisms. Ebola virus infection is mediated following viral attachment, receptor-mediated (co-receptor binding) endocytosis and macropinocytosis fusion mechanisms to invade its host cells and subsequently attacking the innate immune response cells (monocyte, macrophages, natural killer cells, dendritic cells, neurophiles, basophils and eosinophils) and the adaptive immune response cells (CD8 T-cells, CD4 T-cells, B-cells, regulatory T-cells, natural killer T-cells) to evade the immunological response of the host cell. Immuno-evasion and cellular disruption of both immune cells and non-immune cells/tissues remain one of the major hallmarks of Ebola virus infection. The biochemical mechanisms of Ebola virus disease involves rapid viral genomic integration/expression and viremic dissemination of the viral pathogen to other distant cells/tissues; induction of apoptotic signals in both healthy bystander immune cells and non-immune cells/tissues; deregulation in inflammatory response and intravascular coagulation leading to multi-cells/tissue/organs toxicities and eventually death if supportive measures are not adequate to repair and rejuvenate the Ebola virus induced disarray to its host. This review work elucidates the mechanisms of Ebola virus immuno-evasion and cellular dysfunction, geared towards providing an insightful paradigm that can be utilized to combat Ebola virus bio-terrorizing nature.**

Keywords **- Cytopathic effect, Ebola virus, Immuno-evasion, Immunological response, Pathogen, hemorrhagic fever, Zoonotic.**

I. INTRODUCTION

The immune system plays a key role in cytodestruction and detoxification of pathogens in the biological system. Abrogating the immune-surveillance activities in the biological system is one of the trait of viral pathological conqueror to living organisms which effectuate cellular toxicity (cytopathic conditions) and eventually death of an organism. A contagious viral infection of zoonotic origin known as Ebola virus (EBOV), has been reported as a zoonotic viral pathogen capable of inducing cytopathic effect and organismal death by evading host immunological response [1], [2]. EBOV is a virulent pathogen known to cause a highly lethal hemorrhagic fever disease (a disease characterized by bleeding and fever) in human and non-human primates like cynomolgus, rhesus monkeys, African green monkey and baboons [3], [4], [5], [6]. Ebola viruses pertain to the genus Ebolavirus of the family Filoviridae in the order Mononegavirales viruses whose genome consists of a negative-sense with single strand of RNA [7]. Till date, five distinct EBOV species which have been delineated, four species of Ebola virus identified as Zaire Ebola virus (ZEBOV), Bundibugyo Ebola virus (BEBOV), Tai Forest virus (TAFV), Sudan Ebola virus (SEBOV) are known to cause disease in humans and are recognized from Africa [5]. Moreover, a specie characterized from Philippines called Reston Ebola virus (REBOV) is associated with non-human primates and pigs. REBOV strain is considered not being pathogenic for humans but causes hemorrhagic fever in experimentally infected animals [4], [5], [7], [8].

Epidemiological data revealed that The major outbreak of Ebola virus disease (assumed to be the biggest epidemic in the history of the disease occurred in between the year 2013-2016 affecting mostly western African countries (Guinea, Sierra Leone, Liberia, Senegal, and Nigeria) and also spread to other countries in a short span of time was confirmed to be due to a specie known as ZEBOV, as shown in Table 2 and 3 [2], [9], [10], [11], [12], this virus strain outbreak culminated an alarming challenge with an unprecedented scale of more than 28000 confirmed cases and 11000 death and its economic impact on the west African countries was paralyzing [7], [13]. ZEBOV was the first to be reported in the year 1976 causing severe Ebola virus disease outbreaks in Central Africa - Democratic Republic of the Congo with fatality rates ranging from 55% to 88%. In addition, ZEBOV was reported as the Ebola epidemic of the year 2013-2016 in West Africa [14], [15], [16]. For SEBOV, its first and second epidemic were reported in 1970, having its third and fourth epidemic outbreak in 2000 and 2004 respectively with the fatality rates of about 50% for the four epidemics [17], [18]. Tai Forest virus (TAFV) was reported to only cause an illness in one

person who ended up surviving [19]. The Bundibugyo Ebola virus (BEBOV) has it first outbreak in 2007 in Uganda with lower fatality rate of 30%. Moreover, analysis on the genomic sequence of BEBOV reveals that the virus has close relations with the TAFV specie [20]. Reston Ebola virus (REBOV) exist as non-human pathogenic virus and has not been reported in Africa. In 1989, REBOV species caused an outbreak in macaques that were imported in the United States and was later reported in pigs in 2008, as depicted in Table 1 and 2 [21], [22].

Living organisms (e.g. humans and non-human primates) are exposed to myriads of infective organisms, many of which are kept at check by immunological response. The desire to prevent the spread of certain diseases and to develop an efficient treatment against disease outbreak fuelled an early discovery of the immune system [23], [24]. The immune system is made up of antibodies, antigens and many specialized leukocyte (White blood cell) types which is responsible for generating a protective response against infections and invasion by infective organisms (E.g. bacteria, viruses and parasites) and foreign cells (e.g. tumor and transplant) [23]. Immune system is also able to recognize the host cells and molecules and can selectively not respond to self. Innate and adaptive immunity are the major immunological response induced by host organisms against infective organisms or xenobiotics. The innate immunity utilizes macrophages, monocytes, natural killer (NK) cells, neutophils, basophils and eosinophiles whereas adaptive immunity utilizes CD8, CD4 T-cell, B cells, Regulatory T-cells and NK-T cells to mediate immunological response against pathogens [23], [24]. The body immune system operates in a co-ordinated paradigm in responding to numerous threats from the environments which is critical to the survival of living organism beginning from its conception, throughout its development and existence. Living organisms sustain their longevity by employing significant biochemical and immunological surveillance system to identify, neutralizes/detoxify and eliminate pathogens in their system [24]. However, deregulated immune response remains one of the major hallmarks of pathogenic induced tissue damages or cell death.

Ebola virus preferentially attacks the immune cells such as the monocytes, macrophages and dendritic immune cells in order to compromise their host immunological response. In addition to the immune system, Ebola virus also attacks the spleen, kidneys, lungs blood vessels and liver destroying cells/tissues that help the body to maintain homeostasis, regulate metabolic activities, circulation of essential biomolecules, regulate respiratory activities, transport of oxygen to tissues and make proteins that help the blood to clot [25]. Overwhelming both the innate and adaptive immunological activities in the biological systems, Ebola virus destroys blood vessels leaking fluids into the surrounding tissues which result in multi-organ toxicities [25]. Once the virulent Ebola virus form infects the human population, human-to-human transmission of Ebola virus occurs basically via inoculation by injection of the virus into bloodstream or via exposure of mucus membranes or non-intact skin to infectious body fluids or tissues which can result in a major epidemic outbreak in under-resourced settings [7], [26]. Ebola viruses effectuate diseases characterized by rapid systemic viral replication, immune suppression, abnormal inflammatory responses, major fluid and electrolyte losses, and high mortality in human population [7].

Both innate and adaptive (humoral and cellular) immunological response are abrogated upon EBOV infection which produces several viral proteins that inhibits cytokines/chemokine synthesis and response; masking viral epitopes by glycosylation processes; impairing inflammatory response and dendritic cell (DC) differentiation/maturation signals, hence EBOV boost a catastrophic failure against its host immunological response [25]. Ebola virus mutation rate observed is 2.0×10^{-3} substitutions per site per year that is as fast as seasonal influenza, thus posing a serious challenge in developing vaccine against Ebola virus [4]. Ebola virus replicates and transcribed their viral proteins at a higher degree upon invading their host (both in humans and in non-human primates) leading to rapid systemic dissemination of the viral genome and their proteins to multiple cell types inducing myriads of complex pathological conditions such as immune suppression and immune over reactivation in different aspects of the immune response; tissue damage due to direct viral and indirect host-mediated effectors; dysfunctional coagulation cascade mechanism and multi-organ toxicity induction. If adequate supportive care are not met, these processes are known to commonly result in multi-organ failure and death within a short time in humans [26].

Identifying the natural reservoir of Ebola virus over the years remain a major challenge and obstacle in excogitating ways to prevent Ebola virus transmission to humans. However, researchers suspected fruit bats, birds, plants and arthropods as the possible natural reservoir of Ebola virus [27], [28], [29]. Human-to-human, zoonotic or nosocomial transmission of Ebola virus leads to viral infection of the monocytes, dendritic cells and macrophages. Rapid replication and dissemination of the virus through bloodstream to other cells/tissues triggers cells/tissue and vascular damages [26].

Table 1. Outbreaks of Ebolavirus genus between 1976 and March 2016 [5]

EBOV subtype	NO. of transmission	Country	Reported number of cases	Reported number (%) of death among cases	Remarks
Recent outbreaks (since 2014) Ebolavirus	-	Multiple countries mainly three West African countries, namely Guinea, Liberia, Sierra Leone	28,652	11,325	Data between March 2014 and 27 March 2016. Seven countries (Italy, Mali, Nigeria, Senegal, Spain, the United Kingdom, and the United States of America) have previously reported a case or cases imported from a country with widespread and intense transmission.
Ebola virus	1	DRC	66	49	August–November 2014; the outbreak was unrelated to the outbreak of Ebola in West Africa.
Outbreaks between 1976 and 2013					
Bundibugyo virus	2	Democratic Republic of Congo (DRC), Uganda	185	50	
Reston virus	7	Philippines, USA, Italy	13	0	Four outbreaks in monkeys only; one outbreak in pigs with antibody detection in six workers; two out breaks in moneys with seven people developed antibodies.
Sudan virus	8	South Sudan, Uganda, England	779	412	
Tai Forest virus	1	Cote d'Ivoire (Ivory Coast)	1	0	
Zaire virus	15	Republic of Congo, DRC, Russia, Gabon, South Africa	1383	1086	
Total			31079	12922	

Table 2: Chronological data of Ebola virus cases and fatality since it first incidence [5].

Years	Country	Case report	Death	Case fatality rate
1976	Zaire Sudan England	318 284 1	280 151 0	88% 53.1% 0%
1977	Zaire	1	1	100%
1979	Sudan	34	22	64.7%
1989-1990	Philippines	3	0	0%
1990	USA	4	0	0%
1994	Gabon Cote d'Ivoire (Ivory Coast)	52 1	31 0	59% 0%
1995	Democratic Republic of Congo	315	250	71.4%
1996	Gabon	37	21	56.7%
1996-1997	Gabon	60	45	75%
1996	South Africa	2	1	50%
2000-2001	Uganda	425	224	52.7%
2001-2002	Gabon Republic of Congo	65 57	53 43	81.5% 75.4%
2002-2003	Republic of Congo	143	128	89.5%
2003	Republic of Congo	35	29	82.8%
2004	South Sudan Russia	17 1	7 1	41.1% 100%
2007	Democratic Republic of Congo	264	187	70.8%
2007-2008	Uganda	149	37	24.8%
2008	Philippines	6	0	0%
2008-2009	Democratic Republic of Congo	32	15	46.8%
2011	Uganda	1	1	100%
2012	Uganda Democratic Republic of Congo	11 36	4 13	36.3% 36.1%
2012-2013	Uganda	6	3	50%
2014-2016	United State of America Spain United Kingdom Italy Guinea Sierra Leone Senegal Mali Liberia Nigeria	4 1 1 1 3814 14124 1 9 10678 20	1 0 0 0 2544 3956 0 6 4810 8	25% 0% 0% 0% 66.7% 28% 0% 66.6% 45% 40%
Total		31013	12872	41.5%

Structure of Ebola Virus and Its Genome

The Ebola virus (EBOV) structure is made up of three compartments viz; the nucleocapsid, the matrix space and the envelope. Morphologically, Ebola viruses is a membrane-enveloped filamentous virus that is non-segmented, negative-sense with single-stranded RNA of approximately 19kb [5], [25]. When studied under electron microscope, the diameter of Ebola virus filaments is approximately 80 nm and the length is about 14000 nm with 3' nucleoprotein and 5' RNA polymerase end having a spike like virally encoded glycoprotein (GP) of 7-10 nm long projects from its surface of lipid bilayer [4], [5], [25], [30]. Ebola virus nucleotides ranges from 18,959 to 18,961 in length and helically wound RNA genome with seven linearly arranged genes that encodes eight structural proteins [25], [31].

The genome of EBOV consist of seven genes coding nine proteins. The seven genes are sequenced as -3" leader – *nucleoprotein (NP) gene – viral protein (VP) 35 gene - VP40 gene – glycoprotein (GP) gene - VP30 gene - VP24 gene – polymerase (L) gene* - 5" trailer, as shown in Fig. 1 [5], [25]. The conserved leader and trailer regions contain genome replication promoters and packaging signals. Each gene is flanked by 3' and 5' untranslated regions (UTRs) including conserved transcriptional start and stop signals. Most of the genes are spaced by intergenic regions of variable lengths, however certain genes overlap in parts of their UTRs. In addition, all genes are monocistronic with the exception of *GP*, which encodes a total of three glycoproteins. [25], [26], [32].

Nine proteins expressed by the seven genes of Ebola virus are the nucleoprotein (NP); the viral proteins VP24, VP30, VP35, VP40; L (RNA polymerase enzyme); glycoprotein1 (GP1) - attachment protein; glycoprotein 2 (GP2) - fusion/entry protein; soluble form glycoprotein (sGP) - produced from the unedited RNA transcript; and small soluble form glycoprotein (ssGP) – a truncated version of sGP [5], [31], [33]. EBOV surface glycoprotein coded by the GP gene is expressed in two molecular form known as GP1 and GP2 and are generated by RNA editing mechanism [25]. The nucleoprotein (NP), VP30, VP35 and L which are required for the transcription and replication of the viral genomic RNA by constituting a helical nucleocapsid [34], [35]. The glycoprotein (GP), VP24 and VP40 are associated with viral membrane to form the filamentous virions [36]. The matrix protein VP24 has been reported to play a regulatory role in viral genome replication and transcription process, as depicted n Table 3 [35]. GP plays important roles in virus infection and pathogenesis and its expression is tightly regulated during virus replication [37]. The 5'end of the viral genome has about 731 nucleotides and 472 nucleotides from the 3' end sufficient for viral genome replication [4].

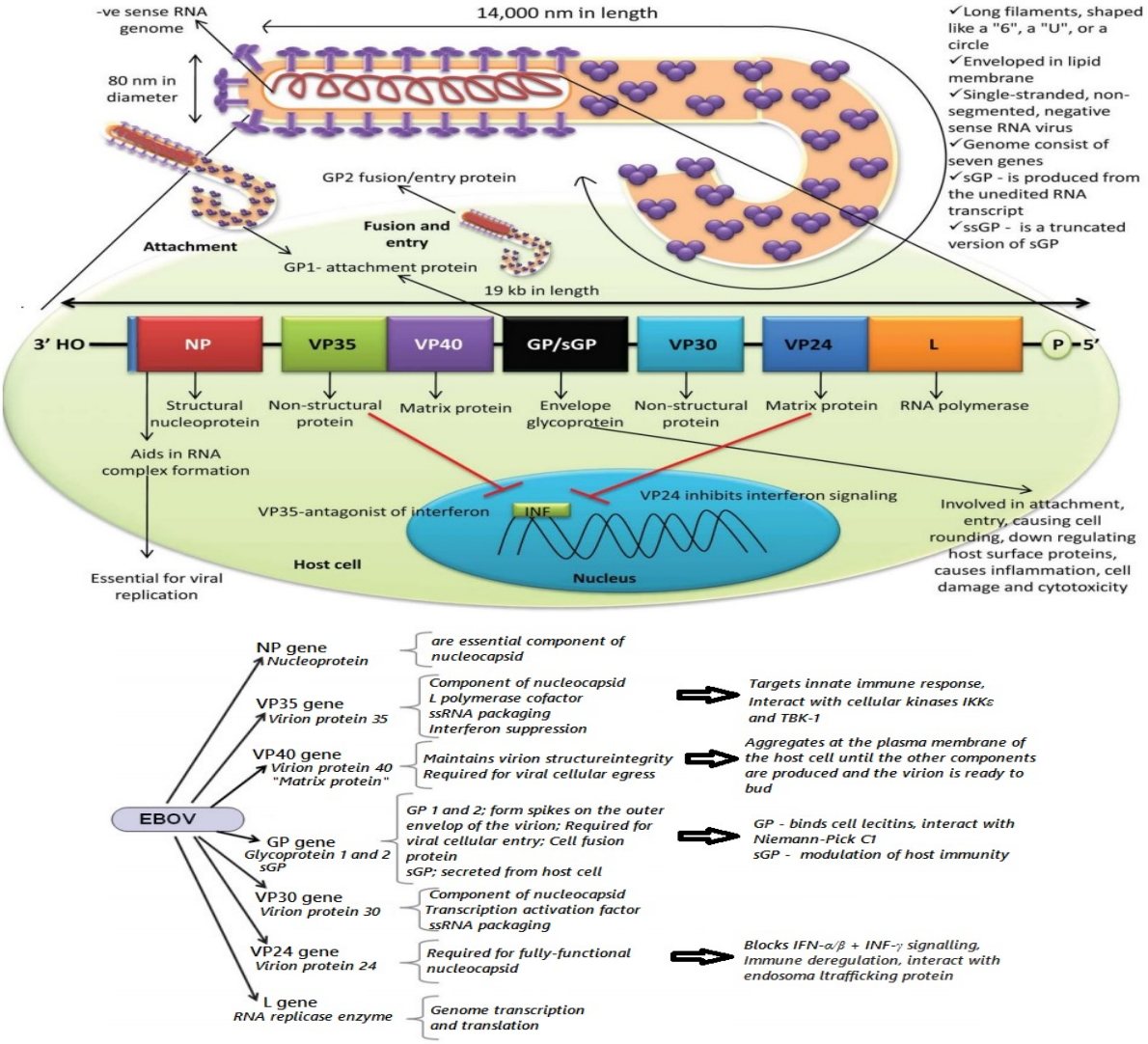

Fig. 1 Ebola virus structure, its genome products and interaction within the host [5], [25]

Table 3.Overview of Ebola virus proteins and the biological roles [2]

Ebola Virus Proteins	Main Function	Secondary Function
Nucleoprotein (NP)	- Key component of ribonucleoprotein complex, encapsidates viral genome and protects viral mRNA from degradation.	Unknown
VP35	- Inhibits type-I IFN signalling by inhibiting activation of IRF-3 via dsRNA binding. - Inhibits type-I IFN production by upregulating SUMOylation of IRF-7. - Impairs dendritic cell maturation. - Counters RNA interference. - Inhibits antiviral effects by blocking protein kinase R.	- Assembly of viral complex with NP and VP30 - Regulates RNA synthesis by modulating NP– RNA interactions and by interacting with dynein LC8.
VP40	- Viral assembly and budding	- Counters RNA interference. - Induces apoptosis in bystander lymphocytes
Glycoprotein (GP)	- Virus attachment and entry	- Late-stage cytotoxicity in cells - Decreases endothelial barrier function - Directly triggers T lymphocyte death and augments monocyte maturation.
VP30	- Initiates Ebola virus transcription	- Counters RNA interference
VP24	- Inhibits IFN-α/β and IFN-γ signalling through interactions with importins, STAT signaling pathways, and NF-κB signalling pathways	- Nucleocapsid assembly and stability - RNA incorporation into VLPs - Regulates transcription and translation
L	- Involved in transcription and regulation of viral genome and mRNA editing	Unknown

Abbreviations: dsRNA = double-stranded RNA; GP = glycoprotein; IFN = interferon; IRF = interferon regulatory factor; L = L-polymerase; LC8 = light chain 8; NF-κB = nuclear factor kappa B; NP = nucleoprotein; STAT = signal transducer and activator of transcription; SUMO = small ubiquitin-like modifier; VLPs = virus-like particles; VP = viral protein.

EBOV Invasion of Host Cytoplasmic Compartment

Research findings reported that Ebola virus enters the target cell by utilizing different uptake mechanisms including lipid raft, receptor-mediated endocytosis and macropinocytosis [38], [39], [40]. Ebola virus adopts three major sequential steps such as viral attachment, co-receptor binding and fusion to gain entry into the host cells. Host cell surface receptor and adhesion molecules exploited by Ebola virus to gain entry to host cells includes β1 integrin receptors; galactose- and N-acetylgalactosamine-specific C type lectin (hMGL); cholesterol-enriched lipid raft micro-domains; dendritic-cellspecific intercellular adhesion molecules DC SIGN-related (DC-SIGNR) and (ICAM)-3-grabbing nonintegrin (DC-SIGN) factors [25], [41], [42], [43]. In addition, different C-type lectins has been identified to enhance Ebola virus entry to the host cells [43]. Ebola virus fusion often utilizes the endocytic routes while exploiting macropinosomes, caveolae and clathrin-coated vesicle pathways to access the host cell cytoplasmic compartment [4].

Ebola virus entry is mediated by the viral spike protein 'glycoprotein (GP)' which docks viral particles to the cell surface receptors on the host cell, as depicted in Fig. 2. T-cell immunoglobulin and mucin domain (TIM) family cell surface receptors bind directly to phosphatidylserine (PS) in the viral membrane. Tyro3/Axl/Mer (TAM) receptors interact with TAM ligands bound to PS in the viral membrane. C-type lectins bind to N- and O-linked glycans present on GP. Following virus-receptor binding, the virus is internalized through a macropinocytosis-like process and trafficked to the late endosome/lysosome where glycoprotein (GP) is cleaved by cysteine proteases, such as Cathepsin B (CatB). After removal of the glycan cap and mucin-like domain (MLD) by cysteine protease cleavage, primed GP interacts with Niemann-Pick C1 protein (NPC1). Subsequently after NPC1 interaction, fusion is triggered and the nucleocapsid is released into the cytoplasm. Current hypotheses for triggering fusion include further cleavage of GP by cysteine proteases and reduction of disulfide bonds by a reductase [25], [44].

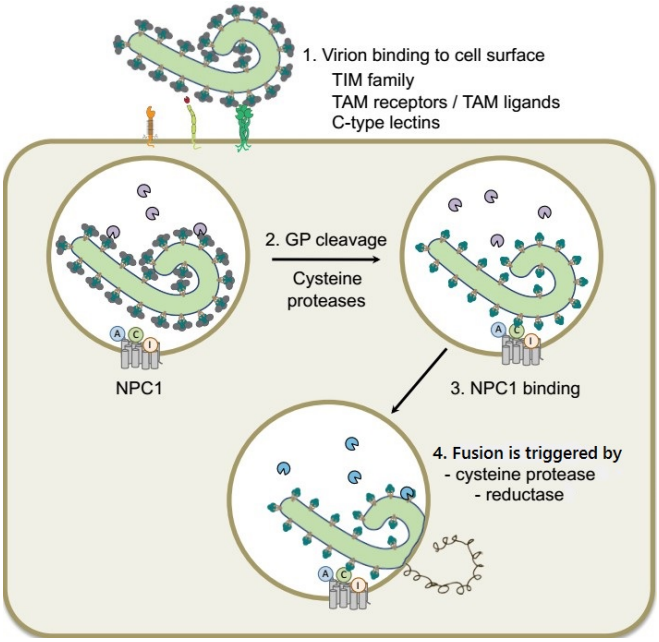

Fig. 2 Mechanistic model of Ebola virus entry into host cell cytoplasmic compartment [44].

EBOV Genomic Integration and Expression

As genomic expressions are tightly regulated following series of gene switch-off and switch-on mechanism in normal cells [45], Ebola virus are capable of integrating its genome in the host genome thereby utilizing the host cell machinery in expressing their viral proteins. Replication and transcription processes of Ebola virus occurs in the nucleus of its host cell involving the interaction of Ebola viral proteins with the host cell proteins, as shown in Fig. 3. Vast arrays of host proteins

such as mucin domain 1, Tyro3 receptor tyrosine kinase family Axl, Nieman-Pick C1, T-cell immunoglobulin, cathepsin L/B, Mer and Dtk have been reported as important cellular proteins utilized in the EBOV entry step [46], [47]. DNA topoisomerase 1 (TOP1) remains the first cellular protein enzyme that contributes to the replication and transcription of the Ebola virus genome, however several other factors involve in this biological processes still remain unknown [48].

Ebola virus coated vesicle fuses into the infected cell cytoplasms where the encapsulated viral genome are released into the nucleus of the infected cell for viral replication and transcription. During replication, the promoter at the 3' 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. The Nucleocapsid proteins (VP35, L, VP30, and NP) associates with the negative-sense genome progeny while GP and sGP undergoes further modification in the endoplasmic reticulum and Golgi body [49]. On the other hand, the Ebola virus genome are transcribed upon insertion into the nucleus of it host cell genome. The RNA-dependent RNA polymerase transcribes individual mRNA from the negative-sense genome in a 3' to 5' direction. Each mRNA is capped at the 5' end and contains a poly-A tail. Subsequently, the transcribed messenger RNA (mRNA) are translocated back to the cytoplasm where mRNA is translated into viral cytopathic molecules or proteins with potentials of budding out, inducing cytotoxicity and terrorizing other healthy bystander cells, as depicted in Fig. 3 [38], [49].

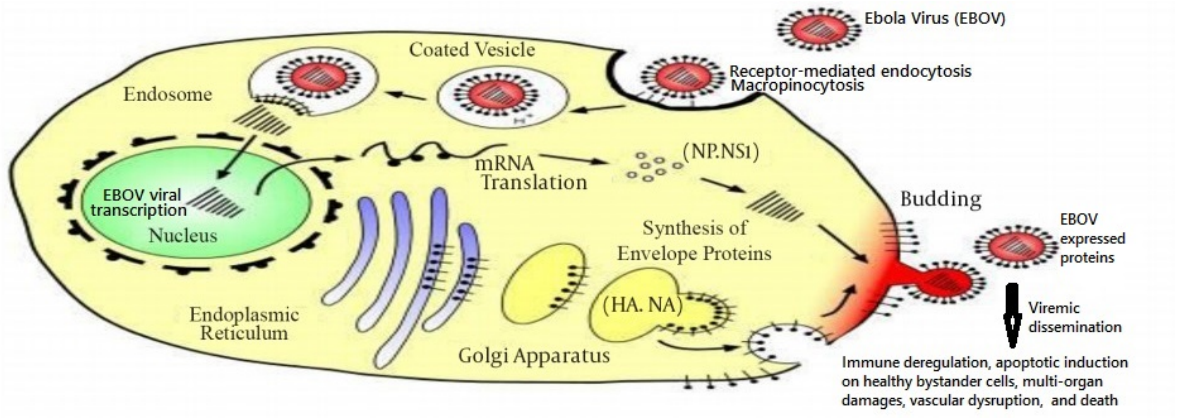

Fig. 3 Ebola virus infection and mode of action [38].

EBOV Transmission, Symptoms and Disease

Contacts with the body fluids of infected person during the treatments, re-using unsterilized medical devices and traditional funeral practicing, kissing, touching and washing the body are the common routes of human-to-human transmission of Ebola virus [50]. Moreover, airborne transmission mode between human still remain unknown. According to Feldmann and Geisbert [51], Ebola virus enter the human body through abrasions, mucosal surfaces and injuries in the skin or by direct parental transmission. Moreover, human-to-human, zoonotic or nosocomial transmission of Ebola virus may directly or indirectly attacks dendritic cells, monocytes and macrophages, deregulating both innate and adaptive immunological response. Rapid replication and dissemination to other cells/tissues triggers tissue and vascular damages, as depicted in Fig. 4 [26].

The incubation period of Ebola virus ranges from 2-21 days with symptoms such as abdominal pain, fever, reduced appetite, diarrhea, vomiting, headache, fatigue, vascular dysfunction, chest pain and shortness of breath. During this condition, fever remains higher than 38.3 °C [4]. Post-Ebola syndrome includes loss of vision and hearing; impotence; bleeding; psychological problems and general weakness [5]. At fatal stage of Ebola virus infection, the innate immune response is abrogated due to massive intravascular B- and T-lymphocyte apoptosis. In addition, there is reduced adaptive immune responses; hemorrhagic diathesis; coagulation disorder and bleeding from organs; shock and multiple organ failure [52], [53]. Survival rate of Ebola virus infected patient is almost zero at fatal stage due to reduced blood pressure because of severe bleeding and loss of body fluids.

Ebola virus disease (EVD) is in humans and non-human primates is characterized by immune suppression; rapid viral replication and transcription of viral malicious proteins; viremic dissemination to distant tissues and organs inducing cells/tissues damages; abnormal inflammatory responses, major fluid and electrolyte losses; high morbidity and mortality rate at fatal stage, as depicted in Fig. 4.

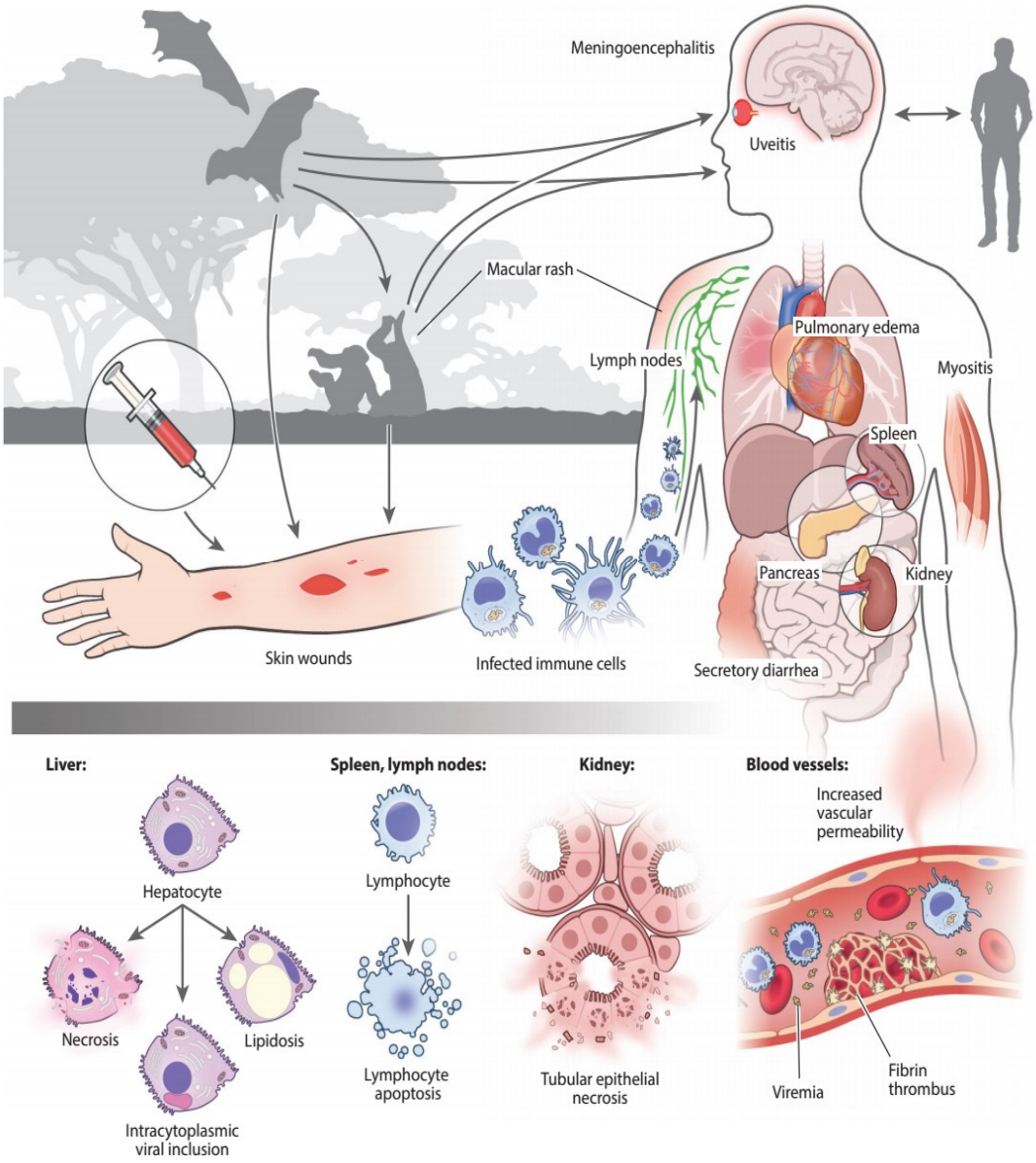

Fig. 4 Overview of Ebola virus transmission and pathogenesis [26]

II. PATTERN OF IMMUNOLOGICAL RESPONSE AGAINST PATHOGENIC INFECTION

The immune system adopts both innate and adaptive immunological response to neutralize/detoxify pathogens (e.g. bacteria, virus and parasites etc.). Innate immune cells react quickly, whereas adaptive immune cells have a delayed response that can take days to fully develop but go on to form immunological memory [23]. The innate immunity cells include macrophages, monocytes, natural killer (NK) cells, neutophils, basophils and eosinophiles while adaptive immunity cells are CD8 T-cell, CD4 T-cell, B cells, Regulatory T-cells and NK-T cells to mediate immunological response against pathogens, as shown in Fig. 5 [23], [24]. Innate immunity is a performed host defense systems that provide immediate host defense activities without first being trained to distinguish self from invader. Elements of the innate immune system often contains structural recognition motifs that allow them to identify likely pathogens to target. On the other hand, the adaptive immune system response against pathogens by generating a specific response to the structure of an invading organism or molecule (antigen) and are also capable of retaining the structure in immunological memory cells for a rapid response and activation for the immune system against an antigen that has previously induced an immune response [23]. However, Ebola virus induces deregulations in host innate and adaptive immunity to elicit its virulent activities.

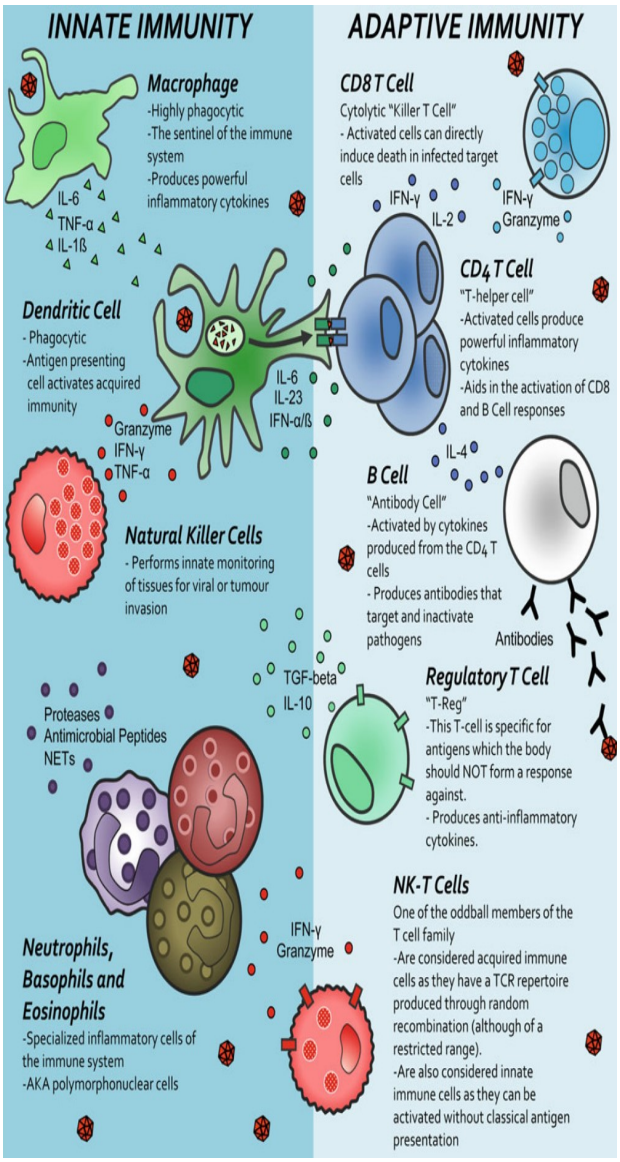

Fig. 5 Overview on innate and adaptive immunity cells response [23].

III. PARADIGM OF EBOLA VIRUS IMMUNO-EVASION AND CYTOPATHOGENIC MECHANICS

Rapid replication (maintaining viral genome), transcription (malicious protein synthesis) and viremic dissemination of Ebola virus upon infecting a host is an effective paradigm adopted by Ebola virus to evade its host immunological response and its subsequent induction of cellular-organismal damages. Ebola virus are capable of coding malicious proteins at various stages of infecting the host to abrogate their humoral and cellular arm defense mechanisms, hence increasing the virulence of the virus. One of the multiple mechanisms employed by Ebola virus to evade host immune response is the antagonism of the type I interferon (IFN) response which is mediated by Ebola viral structural proteins such as VP24 and VP35 [26]. The VP35 induces ribonucleoprotein (RNP) complex production; viral replication and transcription; and impairment of cytokines/chemokine signaling [26], [54]. VP40 matrix protein is involved in the viral budding from the host cell and in the formation of virus-like particles [36]. The envelope GP and other viral proteins are involve in the inhibition processes of the host cell immune response thereby enhancing viral survival and their deleterious activities within its host [4], [55].

Ebola virus utilize diverse mechanisms to establish its complex array of pathogenic events leading to a severe clinical manifestations in Ebola virus disease patient. This can be elucidated from two biochemical perspective which are; 1) direct viral cytopathogenic effects of the virus that causes the destruction of infected cells; and 2) indirect viral cytopathogenic effects which are geared towards amplifying mechanism leading to the destruction/impairment of several crucial body functions such as those induced by the innate and adaptive immune system and by the endothelium [25]. Both innate and adaptive immunological mechanisms are abrogated in the pathogenesis of Ebola virus infection. Innate immune deregulation involves inhibition of type-I interferons (IFNs) response, perturbation of cytokines/chemokines network, functional impairment of dendritic cells (DC) and natural killer (NK) cells while adaptive immune deregulation involves both humoral and cellular-mediated immune arms [25].

Several research reveals that Ebola virus preferable attacks monocytes, dendritic cells (DCs) and macrophages in order to survive and replicate in its host thereby exhibiting its virulent activities [56]. Ebola virus infection of monocytes, dendritic cells and macrophages induces a robust expression of inflammatory mediators - cytokines/chemokines (IL-1β, IL-6, IL-8, MIP-1α, MIP-1β, MCP-1, and TNF-α) [57], [58]. Over expressed levels of these cytokine/chemokines in addition to reactive oxygen and nitrogen species triggers myriads of pathological conditions which have been observed in Ebola virus infected humans and non-human primates [57], [59], [60], [61]. Maturation of DCs is critical in inducing an effective adaptive immune response, however, Ebola virus targets the DCs to impair their differentiation/maturation process which are necessary to stimulate B- and T- cell responses required to destroy pathogens [62].

IV. EBOV EVASION OF INNATE IMMUNE RESPONSE

EBOV infection impairs type-I IFNs production by infected cells and to block IFN response in uninfected cells; induce massive cytokines/chemokines production by monocytes/macrophages; impairs dendritic cell (DC) maturation/differentiation and to deregulate cytokine production; and triggers massive apoptotic activities on natural killer (NK) cells, thus eliminating NK function and inhibiting NK-mediated DC differentiation/maturation as shown in Fig. 6 [25].

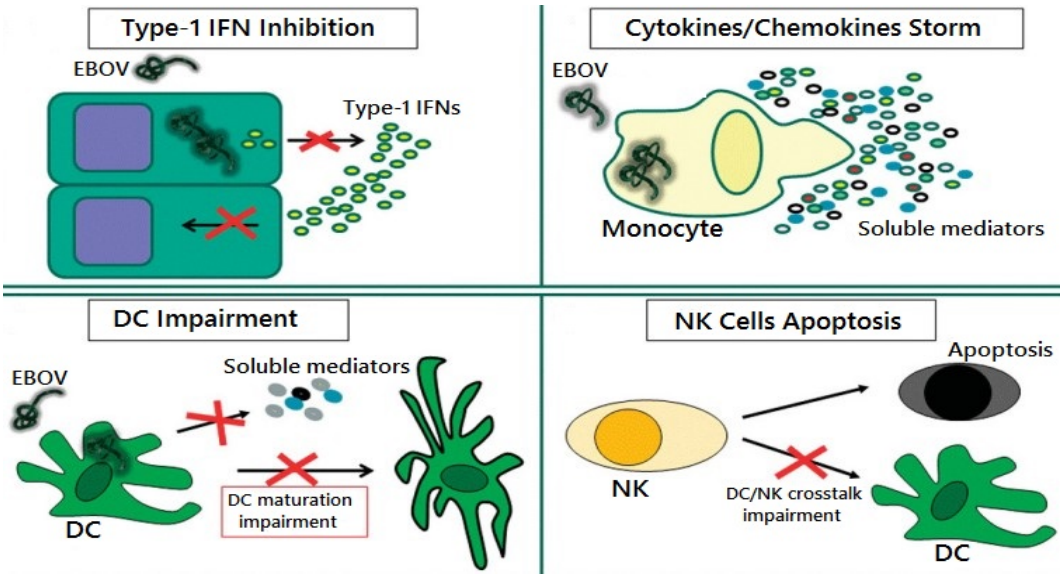

Fig. 6 Ebola virus induction of innate immune cell dysfunction [25].

EBOV inhibition of type-I IFNs response: Type-I IFNs response remains one of the first important innate mechanisms involved in the antiviral immune response. Nevertheless, myriads of both *in vitro* and *in vivo* studies strongly suggest that Ebola virus is able to evade type-I IFNs response (IFN-α and IFN-β) [63], [64]. However, few data reported that early IFN-α production increased the survival rate in both mouse model and in humans [65], [66]. Inhibition of type-I IFNs have been described in Ebola virus infected endothelial cells and the peripheral blood mononuclear cells [25].

Possible proposed mechanisms induced by Ebola virus to inhibit Interferon (INF) include interference with tetherin integrity, steric interference between viral and cellular membranes and exclusion of tetherin from the region of plasma membrane from which EBOV bud [67]. Viral proteins such as VP35 and VP24 have been reported to induce inhibition of IFN production. VP35 suppresses INF-β production via multiple inhibitory effects including the disruption of retinoic acid-inducible gene 1 (RIG-1) pathway by preventing interferon regulatory factor-3 (IRF-3) phosphorylation; the inactivation of IRF-7; and the inhibition of the activation of IFN-inducible dsRNA and Dicer-dependent protein kinase R [68], [69], [70]. VP24 disrupt both type-I and type II IFNs signaling by inhibiting the transcription of antiviral genes. In addition, VP24 specifically prevents the nuclear accumulation of dimerized phosphorylated STAT-1 (signal transducer and activator of transcription protein 1) which participates in both type I (i.e., STAT-1/STAT 2 phosphorylated-dimer) and type II (STAT-1/STAT-1phosphorylated-dimer) signal propagation cascades [71], [72], [73]. Nevertheless, recent research finding reported that residues within the transmembrane domain of glycoprotein (GP) contribute to the inhibition of tetherin activity, a type-I IFN-inducible cellular factor able to prevent enveloped virus budding from plasma membranes [74], [75].

EBOV deregulation of cytokines/chemokines: research findings reported that Ebola virus infection is capable of inducing massive cytokines/chemokine production by peripheral blood mononuclear cells (PBMC) or monocytes/macrophages [58], [76]. The glycoprotein (GP) present on virion surface induces several inflammatory mediator within the first four hours of Ebola virus exposure to the host cell [76]. Cleavage of the virion surface glycoprotein by cellular metalloproteinase produces shed glycoprotein which induces the release of inflammatory mediators. The shed glycoprotein is able to induce the secretion of pro- and anti-inflammatory cytokines by binding and activating non-infected dendritic cells and macrophages mainly via toll-like receptor 4 (TLR4). The recently discovered shed GP activation mechanisms of non-infected immune cells is proposed to have a significant role in systemic inflammation during infection provoking the excessive cytokine storm which are known to be deleterious to survival after infection [25].

Studies on human Ebola virus infection reported that non-survivors expressed high levels of pro-inflammatory cytokines (Interleukin-1β, IL-1RA, IL-6, IL-8, IL-15 and IL-16) and chemokines (macrophage inflammatory protein-1α, and 1β; monocyte chemotactic protein-1; macrophage migration inhibitory factor; interferon-inducible protein-10; growth related oncogene alpha; and eotaxin) that began rising shortly after disease onset and continued to rise until the last sampling within 2 to 3 days before death [60], [77]. On the other hand, survivors of Ebola infection showed an early and short-lived rise in serum cytokines/chemokines indicating innate immune response activation, however fatal infection is associated to a deregulated inflammatory immune response [78].

In addition, Ebola virus infection triggers abnormal production of nitric oxide to induce several pathological conditions such as loss of vascular integrity, tissue damage and apoptosis on

healthy bystander immune cells which is known to play a key role in contributing to virus-induced shock in infected organisms [25].

EBOV induced dendritic cell maturation/differentiation impairment: the rate at which Ebola virus infect dendritic cells (DC) and also exhibit sustained ability to survive for few days are significant in the dissemination of the virus. Ebola virus infected DC are incapable carrying out maturation process and are deactivated from producing cytokines to restore immunological imbalance. However, Ebola virus infected DC induces aberrant DC maturation which is evidenced by upregulation of cell-surface CD40, CD80 and CD83; Increased expression of cytokine, chemokine, antiviral and antiapoptotic genes without significant changes for the expression of lymph node homing receptors or T-cell co-stimulatory molecule genes [79], [80]. A synergistic effects of VP35 and VP24 viral proteins expressed in the Ebola virus infected DC deregulates the infected DC to express aberrant cytokines and chemokines and also induced DC differentiation impairment [62]. DC are inhibited from inducing T-cell proliferation upon Ebola virus infection, this abrogate its adaptive immune response and thereby enhance uncontrolled systemic Ebola virus replication.

Natural killer (NK) cells mediate direct protection against cytotoxicity and also trigger adaptive immune response by helping DC maturation [81], [82]. Nevertheless, Ebola virus infection induces massive loss of natural killer (NK) cells. Thus, the massive NK cell loss in the peripheral blood contribute significantly on the failure to destroy the infected cells and is also responsible for the DC differentiation signal impairment [25].

IV. BIO-MECHANISTIC PARADIGM OF EBOLA VIRUS ABROGATION OF INNATE IMMUNE RESPONSE

The biochemical process involved in Ebola virus subversion of innate immune response are; 1) Ebola virus initially and most desirably infects dendritic cells (DC), monocytes and macrophages. Infection of DCs impairs their differentiation/maturation potentials and suppresses type I IFN responses thereby preventing T cell activation against the pathogen. A robust expression of inflammatory mediators is observed upon Ebola virus infection of the monocytes and macrophage cells. The secretion cytokines/chemokines recruits more monocytes which act as new targets for viral infection. Increased of inflammatory mediators, reactive oxygen species, and nitric oxide triggers lymphocyte apoptotic induction leading to lymphocyte destruction and death. The absent of lymphocytes such as CD4 T cells inhibits the ability of the virus to induce an antibody response. Production of Ebola virus secreted glycoproteins (GP) usurps any GP-specific antibodies that are made. Subsequently, the inflammatory cytokines are responsible for vascular leakage. The Ebola virus systemically disseminates to adrenal glands, kidney, liver and endothelial cells which contributes to symptoms associated with hemorrhagic fever as depicted in Fig. 7 [49].

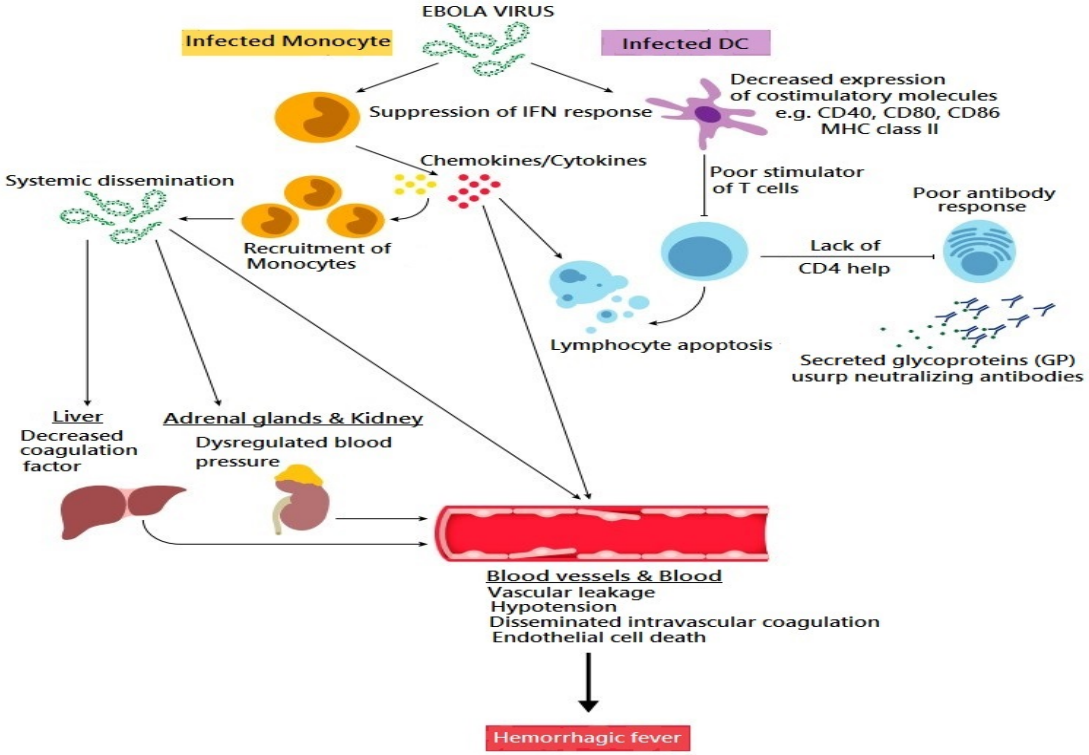

Fig. 7 Biochemical mechanisms of Ebola virus subversion of innate immune response [49].

A vital component for innate immunity against viral infection is the interferon (IFN) response. Under normal immune-biochemical processes upon Ebola virus infection, the viral dsRNA in the cytoplasmic compartment is detected by viral sensors retinoic acid-inducible gene-I and melanoma differentiation associated gene-5. Upon activation, RLRs signal via the adaptor molecule IFN-promoter stimulator-1 which triggers TBK-1 and IKKε kinases to phosphorylate IRFs 3 and 7. Phosphorylated IRF3/IRF7 dimerize and are trafficked into the nucleus where they activate the transcription of IFN-α/β. The IFN-α/β are transported out of the cytoplasmic compartment to bind with the IFNAR1/2 receptor at the plasma membrane where it activates the JAK and STAT signaling pathways. The phosphorylation of JAK1 by tyrosine kinase 2 result in the phosphorylation of STAT1 and STAT2, which then dimerize and translocate to the nucleus, where they activate the transcription of IFN-stimulated genes as shown in Fig. 8 [49].

Moreover, Ebola viral protein (VP35) have been reported to biochemically abrogate the production and cellular response to interferons (IFN) (a vital component for innate immunity against viral infection) by blocking RLR signaling upon binding to dsRNA or PACT thereby inhibiting the production of antiviral cytokines IFN-α/β as shown in Fig. 8 [83], [84]. VP35 can interact with host sumoylation machinery, including SUMO E2 enzyme Ubc9 and E3 ligase PIAS1, to promote the degradation of IRF7/IRF3 [85]. VP35 can also prevent the phosphorylation of IRF3 by IKKε [69], [86]. EBOV VP24 can prevent cellular responses to IFN-α/β by binding to the nuclear importer protein karyopherin α-1 (KPN α1), preventing it from binding to phosphorylated STAT1, thus limiting the accumulation of nuclear STAT1 and preventing IFN-induced gene expression [49].

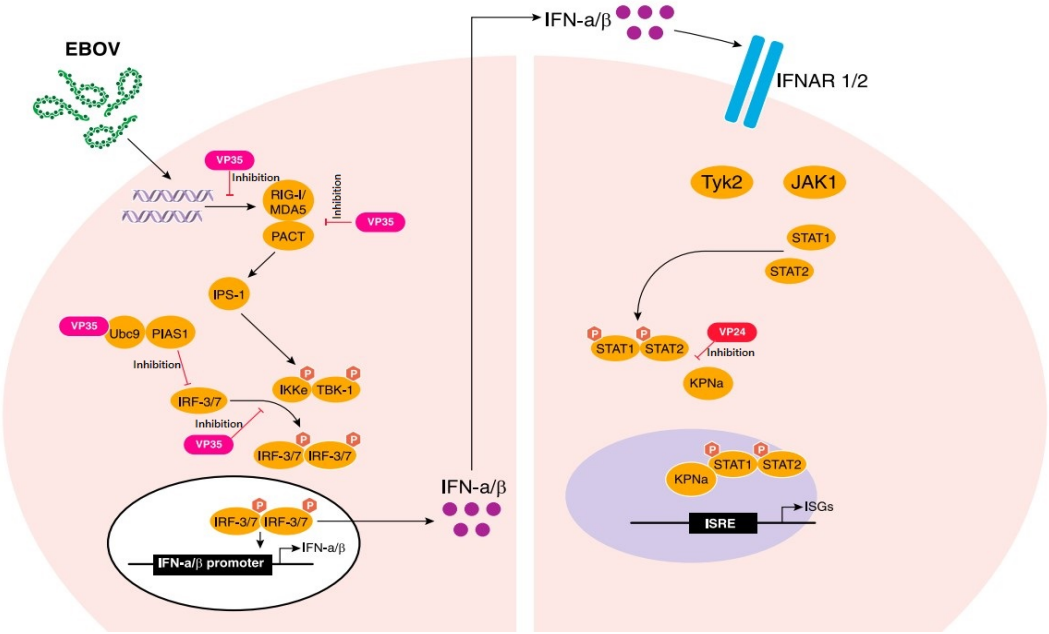

Fig. 8 Mechanism of Ebola virus immune-evasion of interferon (INF) response [49].

V. EBOV IMPAIRMENT OF ADAPTIVE IMMUNE RESPONSE

Ebola virus adopts several mechanisms to evade both humoral and cellular arms mediated immune response. Some of these mechanisms adopted by EBOV include; 1) the ability to produce a GP like chameleon molecules that are able to modulate or misdirect host immune response; 2) shielding the cell free-virus from access to potential virus-neutralizing antibodies by inducing heavy glycosylation on the mucin-like domain of the viral GP; and 3) inducing apoptosis on the immune response cells such as the T-lymphocyte cell [87], [88], [89].

Antibodies production represents one of the best biomechanistic protection during EBOV infection. Two different forms of Ebola virus GP which are soluble GP (sGP) and glycosylated-GP (GlycGP) are able to drive antibodies shielding and misdirection as depicted in Fig. 9. EBOV infection of DC results in a deregulated DC/T synapse, characterized by an effective major histocompatibility complex-peptide/T-cell receptor (MHC-peptide/TCR) interaction (signal 1); a high inflammatory microenvironment (deregulated signal 3); and the absence of co-stimulatory accessories molecules on dendritic cell (DC) surface (ineffective signal 2). The inappropriate DC/T-cell interaction induces T-cell apoptosis and impairs CD4 T-cell clonal expansion, thus blocking all CD4 T-cell helper functions such as CD8-mediated cytotoxicity and antibodies-production by B cells as depicted in Fig. 9 [25].

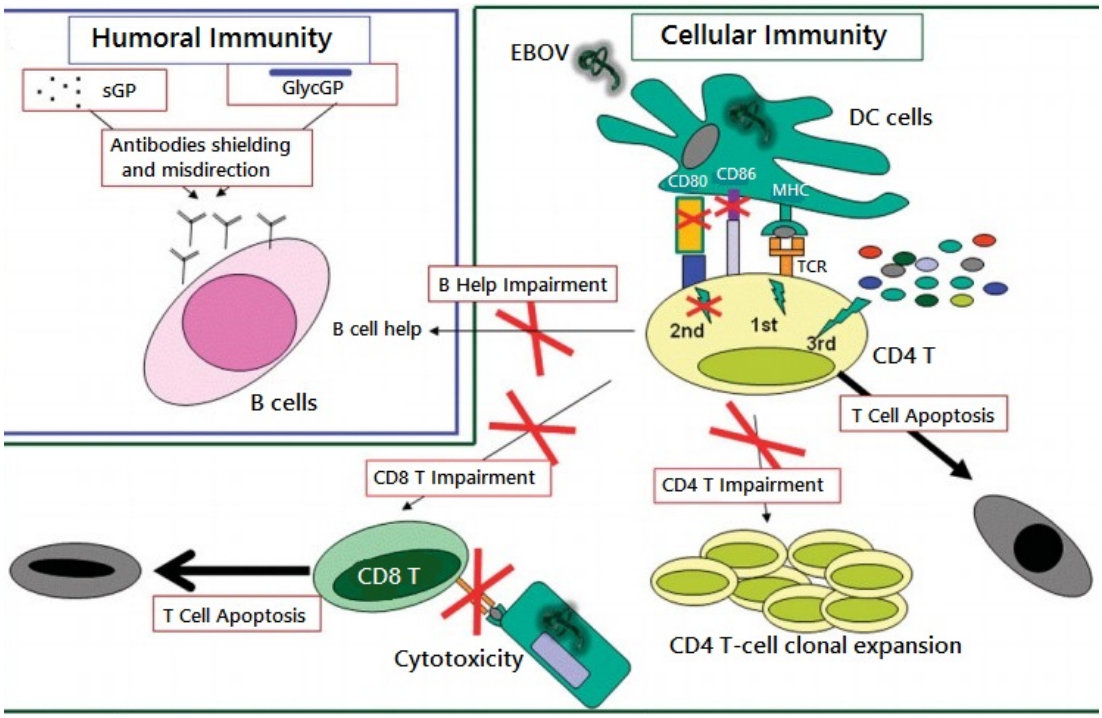

Fig. 9 Mechanistic events of Ebola virus induction of adaptive immune cell dysfunctions [25].

***Lymphocytes apoptotic induction*:** Several findings reported that B and T lymphocytes mediates a protective immune response against viral infections [90]. The activation mechanisms of T-cell against pathogens requires the coordination of certain biochemical signals such as; i) TCR recognition of MHC-peptide; ii) binding of several co-stimulatory molecules between DC and T cells; and iii) balanced ensemble of soluble factors in the microenvironment. A perfect interlinked DC/T-cell interaction of all these biochemical signal is indispensable to CD4 T cell activation which in turn triggers clonal expansion of specific T-cell clone, drives CD8 T-cell cytotoxicity and sustained antibody producing B cells [25]. Nevertheless, Ebola virus infection triggers T-cell apoptosis and DC/T-cell synapses dysfunction. Induction of T-cell apoptosis by Ebola virus inhibits all T-cell helper functions on CD8-mediated cytotoxicity and the production of antibodies by B cells leading to a collapsed adaptive immune response as shown in Fig. 9 [25].

Research finding reported that severe lymphopenia and destruction of lymphoid tissue (loss of peripheral blood $CD4^+$, $CD8^+$, and NK cells) is one of the hallmarks of Ebola infection [91]. 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 Ebola virus patients is an evidence justifying lack of T cell response and destruction upon Ebola virus infection [60], [92], [93]. Over expression of TNF-a from Ebola virus infected dendritic cells, monocytes and macrophages result in increased endothelial permeability leading to vascular leakage [57]. Also, the over expression of nitric oxide being an important effector molecule in the homeostasis of the cardiovascular system can induce loss of vascular smooth-muscle tone and hypotension [61], [94].

VI. EBOV CYTOPATHIC EFFECTS ON NON-IMMUNE CELLS/TISSUES

Ebola virus infection are capable of inducing paralysis of the host response against it survival in other to enhance viral replication and viremic dissemination to non-immune cells/tissues such as the hepatocytes, kidney, adrenal cortical cells and endothelial cells of connective tissue. Liver cell necrosis leads to decreased synthesis of coagulation proteins, while infection and necrosis of adrenocortical cells may negatively affect blood pressure homeostasis resulting in hemorrhage [56]. Tissue damage in Ebola virus-infected individuals is mediated by multiple interrelated mechanisms, including direct viral-induced cytopathic effects and indirect organ injury mediated by host inflammatory responses, endothelial dysfunction, and disordered coagulation mechanism [26].

Ebola virus glycoprotein have been reported as one of the major determinant of pathogenic events in the non-immune cells such as the endothelial cells. Ebola virus GP triggers cytotoxicity and injury on endothelial cells which is characterized by cell rounding and detachment associated by down-regulating cell-adhesion molecules typical of anoikis [95]. Moreover, hepatocellular necrosis have been reported in living organisms infected with Ebola virus, suggesting liver as an important target organ for Ebola virus pathogenesis [96]. The hemorrhagic events observed in Ebola virus infection could be linked to impaired synthesis of blood coagulation

proteins/enzymes resulting in rigorous hepatocellular necrosis [97].

VII. CONCLUSION

The degree of annihilating effects to cells/tissues and organs of both humans and non-human primates induced by Ebola virus infection designated Ebola virus as "a bio-terrorizing agent of zoonotic origin". The biological systems habours myriads of innate and adaptive immune response mechanisms against pathogens. However, Ebola virus infection abrogates both innate and adaptive immunological response exposing the infected cells to both direct viral and indirect viral cytopathic effects, inducing cellular destruction and impairment of several important biological signals that regulates the biological system. Moreover, Ebola virus infection triggers host immune-evasion response to ensure viral replication, transcription of malicious proteins and viremic dissemination of the viral particles and its expressed proteins to distant cells/tissues leading to loss of vascular integrity; multiple tissue/organs damage and apoptosis on healthy bystander immune and non-immune cells leading to death if supportive measures are not adequate to restore the viral cytopathic effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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