Review

Filoviridae: Insights into Immune Responses to Ebolavirus

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Review

Filoviridae: Insights into Immune Responses to Ebolavirus

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Abstract: Ebola virus is a zoonotic virus comprised of 6 different species designated within the family *Filoviridae* and genus Ebolavirus. The first recorded outbreak of an Ebola virus (EBOV) was in Yambuku, Zaire (ZEBOV) in 1976, followed by Sudan Ebola virus (SUBOV) later that year. Outbreaks have been increasing throughout the 21st century, and mortality rates can reach up to 90%. Such extraordinary virulence is evidenced with few pathogens, similarly with Marburg virus (MARV) that originated in Uganda and was first detected in Germany in 1967. The virulent nature of filovirus disease has established these related viruses as a formidable global concern. There are currently four types of *Ebolaviridae* species known to infect humans, with two more recently identified in other animals that are genomically different with respect to cellular pathogenesis or aetiology of disease. Recent advances into understanding the pathogenesis of filovirus disease infections have been remarkable, yet the immunological response to filovirus infection remains unknown. Scientific analysis of cellular mechanisms can provide insight into virulence factors utilised by other pathogenic viruses that also cause febrile illness with occasional haemorrhagic fever in humans. In this review, we aim to provide a brief summary of EBOV proteins and the role of innate and adaptive immune cells known since 2000. We will consider the relevance and implications of immunological proteins measured by CD marker, alongside cytokine, chemokine and other biologically relevant pathways, as well as genetic research. Thorough understanding of immunological correlates affecting host responses to Ebola viruses will facilitate both clinical and applied research knowledge, contributing towards protection against potential public health threats.

Keywords: adaptive; ebola; filoviridae; immunology; innate; molecular

Introduction

Ebola virus (EBOV) is the causal virion of a severe viral induced pathology and disease affecting human hosts through cellular infection and across specific tissues, via transmission through host cell receptors or membranes and intracellular propagation. Total cases of EBOV have amounted to more than 34,626 infections since the virus was first isolated and characterised. Past outbreak mortality was at least 15,266 individuals which is indicative of an infection fatality rate (IFR) of around 50% (range 48%–74%) and the reproduction number (R0) between 2013–2014 was estimated within the ranges 1.51–2.53 [1,2]. Consideration of global incidence of disease or pathology of any description in all age groups is required to clarify research undertaken so far [3]. Despite developments that occurred during the tragic outbreaks in the Republic of Guinea and surrounding areas between 2013–2016 much still remains unclear. However, transmission of zoonotic viruses occurs in other animal species [4]. In 2017, the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (ICTV) was published with updates. Subsequently another species of *Filoviridae* genus named Bombali virus (BOMV) was defined in 2018 not seen in humans currently [5].

Ebola virus is categorised as a Biosafety Level 4 (BSL4) pathogen only researched in designated laboratories (see Supplementary Data). Diagnostics are discussed elsewhere in other articles subject to criteria from the World Health Organization (WHO) protocol and criteria "ASSURED" in order

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to meet the benchmark for usage as point of care (PoC) products to test for any pathogen [6,7]. From research that has been conducted, EBOV virion particle size is estimated at a length of 1–2 μm with diameter in the region of 80–100nm. Each is composed of a negative (–ve) sense single stranded (ss) ribonucleic acid (RNA) genome of around 19kb in size encoding for at least seven predominant proteins. A number of enzymes are necessary for cleavage of host or virion particles for transcription that include polymerases, transferases but also a methyl–transferase. . The interaction between Ebola viral proteins (VP) within a host cell is still being clarified. In general, this family of viruses utilise the RNA genome as a complementary strand to synthesise messenger RNA (mRNA) via the RNA dependant polymerase (RdRp) within a host cell. Viral, bacterial and fungal pathogens utilise different mechanisms for adhesion, permeation, propagation and replication within a host. Other (-ve) ssRNA viruses include the Rabies lyssavirus (Rabies), Hantavirus, Mumps virus (MuV), Measles virus (MeV) and also Rift Valley fever (RVF) phlebovirus that infect different hosts. Currently data on Uniprot suggests that EBOV proteins are observed to be synthesised in rats and mice.

Therefore, here we discuss structure and function of EBOV virion particles, including virus protein components, cellular entry, cellular regulation, along with immune cellular receptor research in context with innate and adaptive immunological mechanisms during EBOV infection.

Method

We performed a comprehensive PubMed literature review encompassing the period from discovery of Ebola virus, in 1976, until 1st July 2023, using the search terms “Ebola”, “Filovirus”, “Cluster of Differentiation”, “Immunology”, “Innate ” and “Adaptive” or in various combinations. Relevant articles to manuscript title were included.

Structure and Function of Ebola Virus

Ebola Virus Protein Components and Cellular Entry

Other reviews summarise current knowledge of proteins known to have been sequenced from the EBOV genome in great depth [8]. Method of transmission of EBOV may be considered to be through bodily fluid and similarly discussed in other reports with sequencing ongoing [9]. Therefore, the scope of this review is to explore knowledge thus far with regards to the interplay between innate/adaptive immune system cells, cytokines (interleukins, IL) , interferons (IFN) and chemokines (denoted by CC or CXC with receptor or ligand (R/L)) alongside cluster of differentiation (CD) molecules [8].

At least seven known EBOV proteins are characterised between the 3’ to 5’ EBOV genomic (-ve) ssRNA strand. In order, these include a helical nucleoprotein (NP), viral protein 35 (VP35), VP40, glycoprotein (GP), VP30, and VP24 with a polymerase (L) protein required for transcription. During 1998 the structure of EBOV GP was further elucidated after prior outbreaks with GP2 appearing to form a trimer with GP1 more important for receptor binding/viral entry requiring a comparatively conserved furin enzyme for cleavage differing between *Filoviridae* with soluble GP (sGP) traversing organelles including the Golgi apparatus and Endoplasmic Reticulum [8,10]. Particle entry of EBOV occurs by forming a ribonucleoprotein (RNP) complex with VP35/VP30 interacting as a dimer and then VP24 followed by the matrix (M) protein VP40 [8,11]. Recently from 2022, reports suggest that VP35 forms a dimer with the polymerase L protein and may underpin the transcription process of the NP as well as replication thereafter [8,11]. It is necessary to correlate the individual size, structure and function of protein composition as immune cell epitope recognition relies on antigen presentation of degraded viral peptides. The EBOV NP protein is estimated at 739 amino–acids, VP35 is 340 amino–acids, VP40 is 326 amino–acids, GP a total of 1,378 amino–acids, VP30 288 amino–acids, with VP24 251 amino–acids and the longer RNA polymerase (L protein) 2212 amino–acids at the 5’ end of the EBOV viral genome [8].

Characterisation of the (-ve) ssRNA EBOV GP occurred before 2009 and brought to light two other glycoproteins (GP1/GP2) together with a small–soluble glycoprotein (ssGP) [8]. The ssGP is described as a di–sulphide linked homodimer and N–glycosylated protein produced through transcriptional RNA editing that can occur of GP1/GP2 with addition of seven uridine (U) residues

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in GP1 and GP2 together with an adenine (A) amino–acid [8,12]. It was previously considered that three biological processes contribute to EBOV virion particle cellular entry. Two methods of virion particle entry were described before 2016 being either clathrin–dependent and/or caveolin–dependent EBOV virion particle cellular uptake. Recently, between 2010–2011 this was clarified as remaining a grey area although logical suggestions were that EBOV utilises micropinocytosis as the main process modifying actin cytoskeletal elements for cellular vesicular entry [13,14]. Thereafter, virion transport occurs intracellularly where GP1 undergoes proteolysis by one of two cathepsins (B/L) eventually leading to GP2 dependent host/virion endosome entry [8]. Further detail is shown below (see Figure 1).

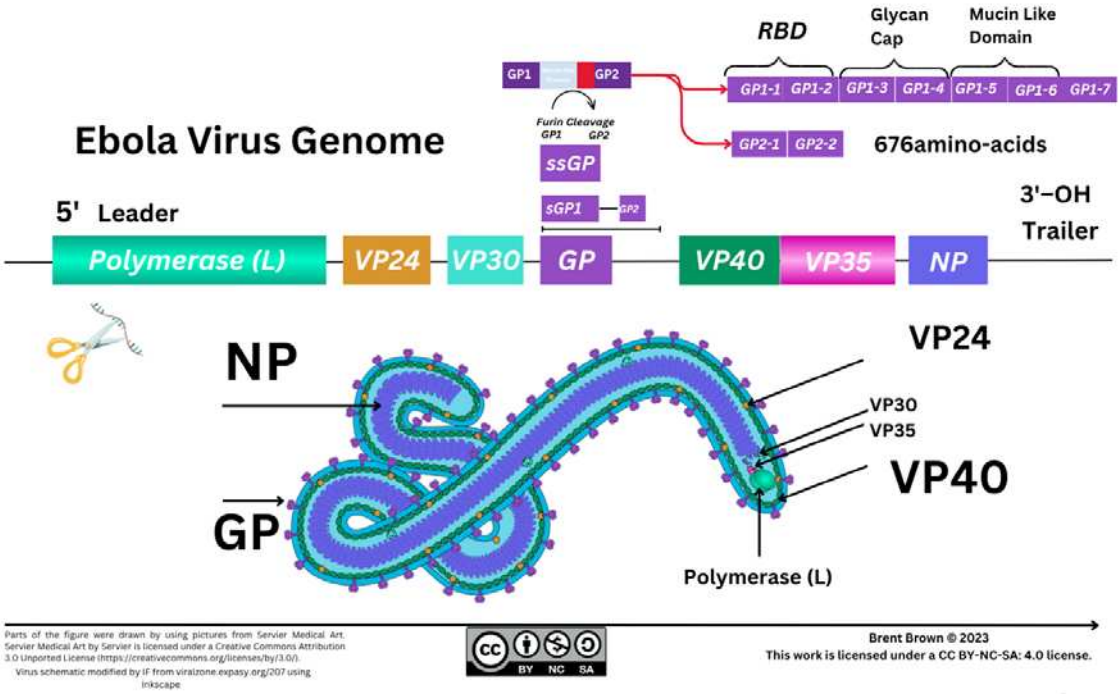

Figure 1. Ebola Virus Genome.

Cellular Regulation and Ebola Virus Proteins

Therefore, cellular EBOV virion–like particles (VLP) produce variable and complex functional proteins within host cells through transcription of viral genome elements affecting parts of the homeostatic cellular compartment with much remaining unknown. For example, EBOV VP35 has amino–acid residues considered to inactivate protein kinase R (PKR) whilst interacting with a protein activator of IFN induced protein kinase (PACT) [8,15]. Predominant functions of VP40 include those of maintaining virion particle structure with significance as a matrix (M) protein; while VP30 role is that of transcription, activation as well as initiation. In comparison, VP24, seemingly interacts with the un–phosphorylated signal transduction activator of transcription 1 (STAT1), but also two types of karyopherin (KPNA1/5) downstream reducing nuclear accumulation of phosphorylated STAT1 [8]. Therefore, it can be considered that a key cellular pathway producing type I IFN (α/β) release is disrupted that trains the immune system response. Many authors concur that the mechanisms of viral EBOV cellular responses are unclear to date [16,17]. Intriguingly, utilising co–immunoprecipitation assays in 2021, it was discovered that the EBOV VP24 protein may interact with emerin (EMD), lamin A (LMNA), lamin B (LNMB) as well as lamin C (LMNC). It was highlighted and seen each protein was required to stabilise nuclear membranes that are disrupted [18–20].

Prior articles investigating EBOV GP mutations, specifically within A82V, are relevant and may or may not enhance cellular virion particle entry *in vitro* of the EBOV glycoprotein [21]. Nevertheless, it was highlighted *in vitro* that an amino–acid substitution where alanine was substituted with valine,

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as both contain neutral non–polar R groups and a methyl (CH3) addition, that an increase in EBOV cellular infectivity was observed [21]. Moreover, a change between threonine to isoleucine at residue 544 of the glycoprotein (GP) increased virion particle kinetics during viral host plasma membrane (PM) fusion utilising the intra–cellular Niemann–Pick type C1 (NPC1) receptor within endosomes [21]. The crystallised protein structure of NPC1 was observed with clarity that a pocket exists within the cholesterol transport pathway where low–density lipoproteins (LDL) are processed [22]. Thereafter, *in vivo,* NPC1 was observed in deficiency studies, where EBOV was non–contagious with EBOV GP appearing to form a complex with the NPC1 luminal domain . One enzyme, a serine cathepsin protease L, was considered to be more important in proteolytic cleavage enhanced by the virion EBOV GP around or within the endosome. In order to replicate, the RNA polymerase transcribes from the 3′ trailer to the 5′ leader genetic viral RNA sequence intracellularly. This occurs through capping and adenylation utilising the L protein RNA polymerase sequentially but also through RNA editing to express full–length glycoprotein (GP). Ebola virus replication through endosomal pathways remains under research investigation [23]. The exact mechanism EBOV utilises to form intracellular late endosomes remains unknown. Recently VP40 is ascribed to be crucial in membrane fusion during endosomal disassembly within cells (see Figures 2/3) [24].

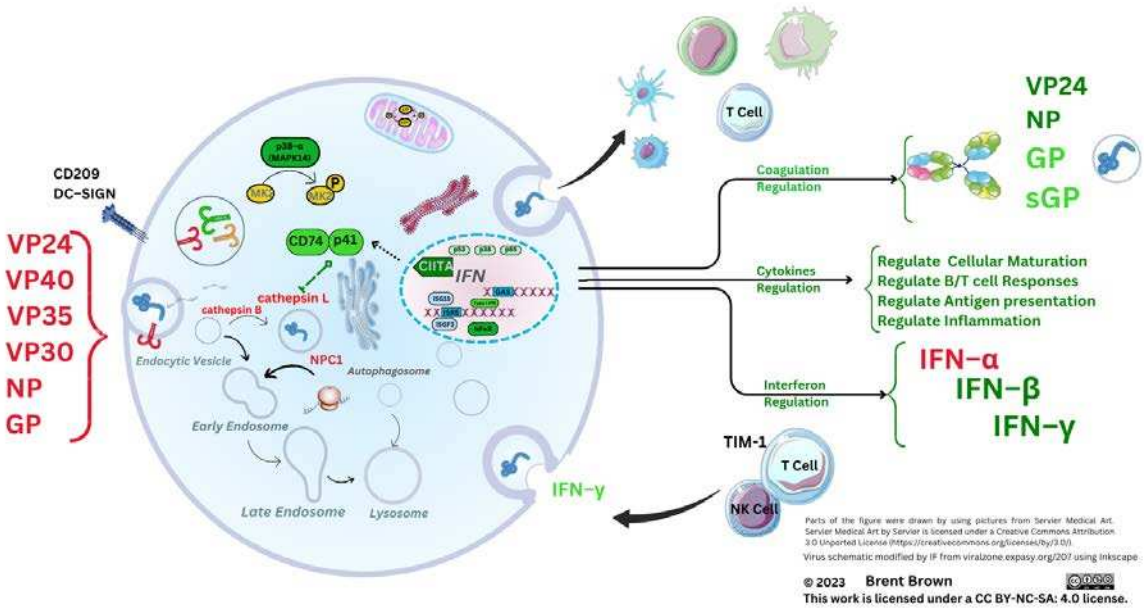

Figure 2. Ebola Virus Cellular Pathways.

Immune Cellular Receptor Research during Ebola Infection and Disease

Viral entry occurs by attachment to host cell membrane receptors or disruption of the cell/ PM utilising either receptors, ion channels, adhesion mechanisms or attachment factors for permeation. It is considered that the EBOV GP utilises a C–type lectin DC–SIGN (CD209) receptor expressed by dendritic cells (DCs) and macrophages (Mϕ) as well as other host cells that facilitates virion particle entry. The corresponding receptor is a DC specific intercellular adhesion molecule–3 (ICAM–3/CD50) receptor. Data on proteinatlas.org shows DC–SIGN (CD209) is differentially expressed by host cells but at increased levels throughout the gastrointestinal (GI) tract, bladder, heart muscle and within the lymphoid organs that are the thymus, spleen as well as lymph nodes. It is also present in smaller quantities within the brain (e.g. cortex, hypothalamus etc.), but specifically within the choroid plexus. Current understanding of DC–SIGN evolved from 2000 research when it was ascribed that DC–SIGN and a homologous protein L–SIGN (CD209L) receptor both had mannose binding motifs and are calcium dependent and bind with high affinity to mannose N–glycans present in pathogens [25]. Shortly after, between 2001–2003, indications were that this receptor was required for *Mycobacterium tuberculosis infection of DCs and CD209 may potentially be utilised with ICAM–3 (CD50) present on T cells*

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as an immunological synapse necessary for priming naive T cells [26,27]. In addition the DC–SIGN receptor (CD50) is suggested to have high avidity for EBOV GP with both binding to CD209 [28].

From 2006, emerging *in vitro* research indicated a synthetic peptide that could affect (inhibit) two types of *Filoviridiae* (MARV/EBOV) by activation of neutrophils. Activation of neutrophils during EBOV is intertwined with the pro–inflammatory response occurring through triggering receptors expressed in myeloid cells (TREM–1) regulating viral induced increases in intracellular calcium as well as reducing secretion of cytokine tumor necrosis factor–α (TNF–α) [29,30]. Circulating neutrophils are central to both endothelial cell (EC) adhesion, and platelet aggregation as well as activating other immune cells and also secret serine proteases (e.g., cathepsin G, elastase) and are sensitive to IL–6 due to high expression of the IL–6Rα/gp130 receptor sub–unit domain [30–32]. It was indicated then that TREM–1 could be shed from the PM whilst increasing phosphorylation of decay activating proteins (e.g. DAP–10/DAP–12) possessing a conserved immunoreceptor tyrosine–based activation motif (ITAM) [33,34]. Since discovery, DAP–12 protein is described as a signaling adaptor molecule containing aspartic acid within the PM forming stable non–covalent homodimers that may interact with a number of natural killers (NK) cell receptors like CD94/NK2GC/E but also CD158 and other immune cells [33]. Gene transcripts and proteins of DAP–12 have been described as in abundance in pDCs, monocytes, Mϕ as well as NK cells with lesser amounts present in as yet unknown sub–types of $\alpha\beta$ T cells expressing a T cell receptor (TCR) known as $CD4^+$ and $CD8^+$ T cells [33]. Signaling regulation occurs through c–Jun N–terminal kinases (JNKs) but also spleen tyrosine kinases (Syk) in myeloid cells [33]. Further to this a homologous zeta–chain–associated protein kinase of molecular weight 70kDa (ZAP70) was identified as a crucial CD3 TCR expressed during T cell maturation [35]. Interestingly Syk is not expressed in T cells but ZAP70 is expressed in NK cell and T cell populations with research ongoing. The importance of ZAP70, a cytoplasmic tyrosine kinase, can be considered, as ITAM tyrosine residues can be doubly phosphorylated; thereafter resulting in signaling through ZAP70 with high affinity through –SH2 domains within the locality of the TCR/MHC class II complex required for antigen/peptide dependant TCR signaling [33,35–37].

Prior to 2011, reports emerged investigating other receptors further clarifying unknown mechanisms of EBOV cellular PM entry. The T–cell immunoglobulin–mucin–domain 1 (TIM–1) were of consideration, denoted as CD365 *in vitro*, that may facilitate EBOV cellular infection in overall *Filoviridae* research [38]. Mucosal epithelial expression of TIM–1 was suggested to be a potential receptor for EBOV cellular infection and route of entry. Recently, TIM–1 is suggested to also facilitate late viral virion particle egress. In 2017, adaptive T cell studies investigating the immune response further clarified that TIM–1 is a relevant attachment factor for EBOV together with a further three attachment factors that are, L–SIGN, folate receptor–α and Tyro3 receptor tyrosine kinases [39,40]. Conundrums remain, as during 2019, TIM–1 would appear to *in vivo* not affect overall mortality, although as discussed further, EBOV stimulation affects T cell receptors with TIM–1 (CD365) affecting cytokine regulation. As research evolved, deletion of the mucin domain of EBOV GP1–5 has some effects unknown so far and may hold future promise with chemokines emerging that include CXCL10 and CCL2 and others of note in immunological responses detailed further below [40–42].

It is indicated that the EBOV VP40 protein requires phosphatidylinositol 4,5–bisphosphate ($PI(4,5)P_2$) and phospatidylserine (PS) forming oligomers at the PM surface also facilitating vesicular particle egress [43]. Recently this was followed up to note VP40–membrane binding via electrostatic interactions with cationic VP40 residues. Therefore VP40 interaction with lipid residues was further clarified with phosphatidic acid (PA) as a key part of formation of EBOV M proteins [44]. It is of note that phosphatidylinositol (PI) is phosphorylated at the PM surface into $PI(4,5)P_2$ and requires PA but also protein lipase C (PLC) and can hydrolyse phospholipids whilst $PI(4,5)P_2$ regulates actin formation required within epithelial layers an important EBOV method of transmission [45]. Other receptors remain unclear, although considered prior were fibronectin receptors (denoted by integrin sub–unit domains) present within the extra–cellular matrix(ECM) (e.g., $\alpha5\beta1$). As discussed, NPC1 protein is a 1278 amino–acid internal cellular receptor localised to endosomal and lysosomal membranes and in 2015 was confirmed to be essential for EBOV replication and pathogenesis *in vivo* [46]. These were notable findings, because NPC1 is uniformly expressed throughout the brain but

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preferentially within plasmacytoid (pDC) dendritic cells and endothelial cells (ECs) of the vascular system and possibly at decreased quantity within other immune cells. The unique receptor that EBOV utilises remains unknown, but viral EBOV GP is required for micropinocytosis and vesicular transport within cells [13,14]. Furthermore, the ECM may contain other short signaling peptides unknown to date acting as cryptic pockets that differential EBOV proteins interact with triggering structural interaction allowing differential EBOV binding to cellular receptors [47].

During 2011 it was further elucidated *in vitro* that VP24 disrupted a nuclear p38 mitogen–activated kinase (MAPK) gene transcript (*MAPK14*) and the resultant enzyme synthesis [48]. It was distinguished that MAPK p38–α disruption was one key factor in type I IFN–β inhibition; but also that EBOV VP24 binds to karyopherin A1 (KPNA1) while inhibiting phosphorylated signal transducer and activator of transcription–1 (STAT1) nuclear accumulation. Therefore, as KPNAs would usually transport proteins through nuclear pores, the resultant STAT1 signaling stimulating IFN synthesis through interferon stimulatory genes (e.g. *ISG15*) could be affected by different EBOV virion particle sub–unit domains [48]. Mitogen activated protein kinases are serine/threonine–protein kinases regulating p38 cell cycle progression. Disruption to p38–α regulation between the growth and mitosis cell cycle (G2/M) could therefore plausibly be considered; because p38 may regulate stability and translation of both TNF–α and IL–6 affecting neutrophil migration and can be expressed by affected endothelial/epithelial cells [49]. Furthermore, it is apparent that the deletion of p38–α within DCs *in vivo* has consequential effects on DC function. The p38–α deletion can result in changing viral antigen presentation to cytotoxic T (T_C) cells expressing CD8 from conventional DCs (cDC) and reducing antigen presentation ability of $CD8^-$ cDCs accompanied with reduction of DC maturation cytokines IL–12p40 and IL–12p70 [50]. Therefore, viral EBOV mRNA may in effect dysregulate cytokine production as well as nuclear cell cycle regulation and transcriptional regulation affecting antigen presentation to T cells remaining unknown to date (see Figure 3).

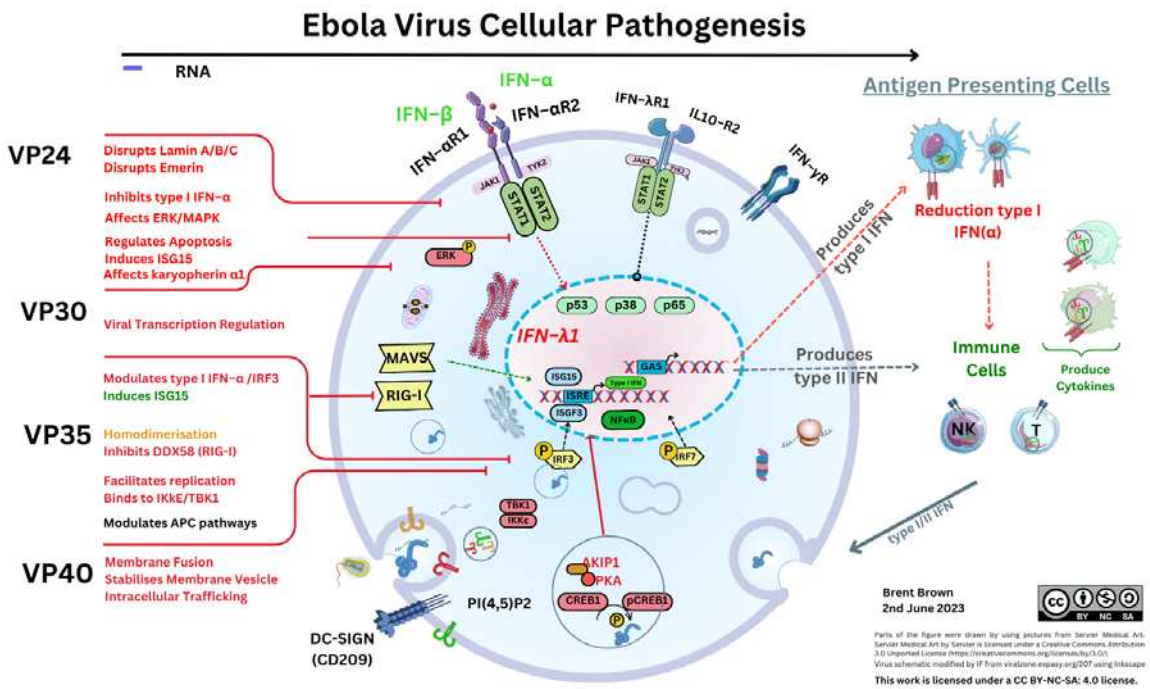

Figure 3. Ebola Virus Pathogenesis.

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Innate and Adaptive Immunological Mechanisms during Ebola virus infection

Overall Perspectives and Antigen Presenting Cells

During 2006, early research articles implicated the GP relevant to *Filoviridae* pathogenesis as development and results allowed more insight whilst genomic sequencing technology improvements occurred. At this time within the Marburg viruses whole–genome sequencing identified the existence of a Ravn virus (RAVV) characterised [51]. Initial immunological research investigating *Filoviridae* was suggestive that an imbalance in helper T cell (T_H) and cytotoxic T cell responses may occur. The combined interaction of innate and adaptive regulation of the T cell response remains a topic requiring further detail. It is known that optimal immune responses require B cell antibody production; but also T cells alongside type I/II/III interferon (IFN) synthesis and require a homeostatic cytokine/chemokine balance to function in an autocrine/paracrine systemic manner. This in effect regulates anti–viral, anti–bacterial and anti–fungal activity through cytolytic actions and phagocytosis by antigen presenting cells (APCs), T_C cells, and NK cells. In our last article we discussed antigen presenting cells (APCs) encompassing DCs, monocytes and macrophages (M1ϕ and/or M2ϕ) by CD molecule expression [32]. These cells present and process pathogen derived peptide epitopes to T cells utilising major histocompatibility complex (MHC) class I/II molecules. Dendritic cells are considered to have a unique tolerogenic profile. This cell population has the highest expression of MHC class II molecules and presents EBOV antigens [52]. Monocytes are classified as non–classical, intermediate or classical with varying functions including pathogen phagocytosis. Macrophage categorisation is intertwined with cellular polarisation and upregulation / downregulation of various CD markers . Recently these are referred to as M1ϕ or M2ϕ referring to predominant pro–inflammatory or anti–inflammatory. During 2017 it was designated that four EBOV proteins could potentially be key immunogenic proteins in positive innate and adaptive immunological responses, namely VP24, NP, GP ,and sGP [53]. Below is shown some of the known EBOV protein function to this point with some of the relevant observed gene transcripts and cytokines observed during infection and within laboratory research hitherto that affect IFN synthesis and coagulation regulation pathways (see Table 1) [8,54].

Table 1. Ebola Virus Proteins and Gene Regulation Factors.

Proteins Encoded	
NP	Nucleoprotein
VP35	RNA-dependent RNA polymerase (RdRp) cofactor Inhibits type I IFN
VP40	Matrix Protein
GP	Soluble secreted glycoprotein (sG)
GP	Δ-peptide
GP	Full length spike GP
GP	Soluble small secreted glycoprotein
VP24	Nuclear membrane disruption Inhibits type I IFN
L	Polymerase

Viral Host Restriction Genes	
Tetherin	*Antagonist conserved in filoviruses*
ISG15	Affects Interferon Synthesis
STAT1/2	Affects Interferon Synthesis
IFITM1	Affects Interferon Synthesis
CSF1	Affects Laminin / Emerin

Host Dysregulated Protein	
Thrombospondin	Coagualant Regulator
Serpin B2	Inhibitor of urokinase-type PA

Host Genes Expressed	
THBD	*Thrombospondin*
SERPINB2	*Serpins*
AKIP1	*A Kinase Interacting Protein 1*

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Initial immunological laboratory observations are researched and measured by serum cytokine concentration changes during infection often referred to as a "cytokine storm" in an autocrine/paracrine dependant manner in conjunction with chemokines and Toll–like receptors (TLR) with a 2015 CD classification update on all relevant immune cellular markers [55]. Study of these can explain some of how the cellular/molecular immune system synchronises during health and disease with much remaining unclear [56–58]. During 2003, the predominant APCs were further investigated with regards to *Filoviridae* infection. Dendritic cell research has evolved since 2017 [59]. Accordingly the profile of DCs remains a mystery in EBOV infection amongst other viral diseases that is dependent on a multitude of transcription and activation factors . These contribute towards the tolerogenic and anergic profile of DCs. Since 2017, DCs are broadly classified into non–classical plasmacytoid (pDC), but also a further three classical/conventional sub–types (cDC1, cDC2 and cDC3) [32].

In 2003 it was indicated that EBOV VP35 could infect DCs of the monocytic lineage affecting the T cell response [60]. It was then observed through *in vitro* culture that DCs maturing into a monocytic lineage secrete IL–1β, IL–6 together with IL–8 and express CCL5 (RANTES) with abrogation of type I IFN-α that is usually secreted in high quantities [60,61]. More recently it was confirmed that DCs obtained from EBOV peripheral blood mononuclear cell (PBMC) derived monocytes (moDCs) could permit EBOV replication but pDCs did not [60]. Importantly a generic leukocyte expressed marker, $CD45^{+}$,was used to determine the cellular lineages as either MoDC ($CD11c^{+}CD16^{-}CD14^{-}$HLA-$DR^{+}$) or pDC ($CD11c^{+}CD14^{-}CD16^{-}CD123^{+}$HLA–$DR^{+}$) expressing human leukocyte antigens (HLA–DR) that are required to present peptides to T cells (see Figure 3) [60]. With research ongoing since it was confirmed that several key immune cell check-point gene transcripts (*CD40, CD80, CD83*) were potentially affected in DCs by EBOV during the 2013–2016 outbreak with clarity that VP35 could unblock antigen processing pathways and IFN transduction [48,62]. Uniquely, it was seen in a key study each of the type I/III IFN gene transcript changes in dendritic cells [52]. These indicated that during EBOV infection that VP24 may counteract earlier VP35 antagonism of DC IFN transcription of gene between 8–24 hours after infection and up to 5 days later [52,63]. Type I IFNs are usually required to activate and regulate both Mϕ, NK cells and the T cell effector response [64]. The significance of this still remains unclear.

Table 2. Cytokine Regulators during Ebola Virus Infection.

PBMCs		
Cytokine	**IFN**	**TNF**
IL-1β	IFN-γ	TNF-α
IL-10	IFN-α2	
IL-18		
IL-12p40		

Seminal *in vitro* reports prior to 2013 also clarified some of the mystery investigating monocyte cell roles during EBOV infection. It was indicated that monocytes could be permissive to infection after a delay up to 48 hours in effect allowing EBOV cellular replication. Key differences were clarified with preferential mRNA expression in monocytes of three IFN regulatory gene transcripts (*IFITM1, IFITM2, IFITM3*) that in effect regulate intra–cellular viral replication cycles through IFN synthesis, while NPC1 was distinctively expressed in pDCs at higher concentrations than within monocyte derived lineages usually presenting viral antigenic epitopes to T cells [65]. It is therefore observed that

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EBOV can infect two APCs (Mϕ and DCs) with EBOV VP24 and VP35 having dual effects modulating overall duration of leukocyte type I IFN synthesis and secretion of which the significance is unclear [48,52,62].

During EBOV infection, early indications were that a change in secretion of type II IFN–γ may occur usually secreted by T cells and NK cells. This was accompanied by reduced IL–12 and IL–2 synthesis with increased IL–10 and increased T cell apoptosis within 12 hours upon cellular stimulation [60,66]. Synthesis of IL–12 is crucial (see Figure 4 below) as signaling occurs through p35/p40 sub–units effecting DC responses in synergy with IL–23 and other cytokines via the heterodimer p70 formed with IL–12R (IL–12Rβ1/IL–12Rβ2) and a heterodimer shared with IL-23 [67,68]. In 2015, this was examined further to find exogenous stimulation could *in vivo* inhibit EBOV replication within Mϕ to find four chemokines expressed that include CXCL9, CXCL10, CCL8 and CXCL11 together with complement proteins (C1s and C1r) both of which are usually regulated by IFNγ and three of these have a receptor (CXCR3 (CD183)) on DCs [69–71]. Moreover, upregulation was noted of the Mϕ endocytic marker (CD163) shed from the PM localising with viral EBOV antigens around Mϕ and hepatocytes [72]. The glycoprotein CD163 is also expressed by DCs during maturation. It is a haemoglobin scavenger receptor specific for both monocyte and M1ϕ/M2ϕ lineages and facilitates uptake of haptoglobin/haemoglobin (Hp–Hb) complexes in the circulation where lysis of erythrocytes occurs during EBOV infection. It was further noted in 2006 that lipopolysaccharide (LPS) stimulation of TLR4 also causes CD163 shedding in monocyte lineages with TLR2 and TLR5 present at the cell PM surface [73–75]. Recently (2017), TLR4 studies *in vivo* and *in vitro* in the absence of T cells are indicative that EBOV activates TLR4 with each of the CD11b⁺ and CD11c⁺ lineages (DCs, monocytes, M1ϕ and neutrophils) circulating in draining lymph nodes (dLNs) [76]. Further to this observation, using GP stimulated bone marrow derived Mϕ (BMDM) it was observed *in vivo* that TNF–α, IL–1β, and IL–6 normalised within 24 hours; moreover CCL2 and CCL4 together with T cell–derived cytokines (IL–2, IL–4, IL–5, IFN–γ) and IL-10 remained elevated. Noteworthy was that CCL5 (RANTES) and IL–12 expression/synthesis remained unchanged. However *in vivo* studies in detail indicated Mϕ cells continue to upregulate two co–stimulatory molecules (CD40/CD80) and potentially could still signal to three respective T cell ligands (CD40L/CD28/CTLA4) which are required for adaptive T cell responses.

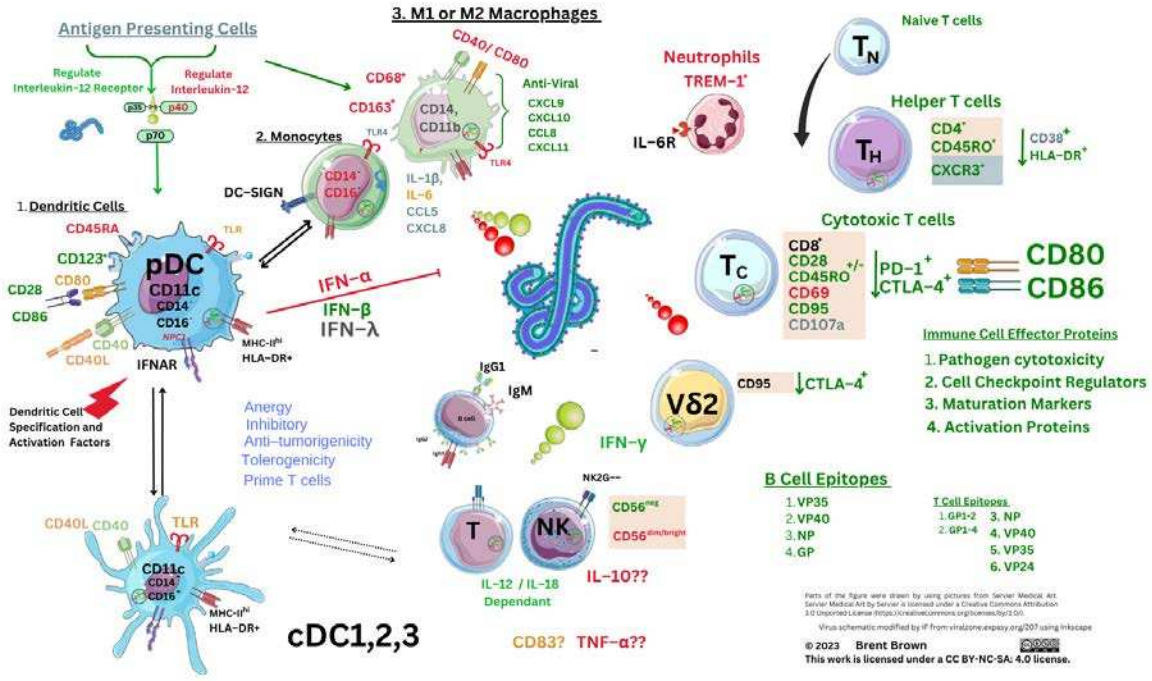

Figure 4. Immune Cellular Response Profile by Cluster of Differentiation (CD) molecule during EBOV infection.

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From 2016, further detailed analysis occurred *in vitro* comparing EBOV and MARV of consideration as both are classified as *Filoviridae* [77]. This laboratory utilised cellular transcriptome mapping *in vitro* to note upregulation of an annexin gene transcript (*ANXA3)* with the potential protein encoded known to inhibit phospholipase 2 (PLA2) enzymes [77,78]. These were key observations as PLA2 is crucial and metabolised by cyclooxygenases (COXs) into anti–inflammatory mediators including leukotrienes (LTs), prostaglandins (e.g., PGE2) as well as eicosanaoids. Also observed were *IL–8* and *IL–32* that could affect T cell apoptosis. Another gene transcript, *CYR61*,was observed known to encode a known cysteine–-rich protein binding to heparin and is a growth factor (GF) affecting vascularisation. The dual specificity protein phosphatase 1 *(DUSP1)* gene transcript was highlighted encoding a protein processing intrinsic phosphatase activity that can inhibit the extracellular signal–regulated kinase (ERK1/2/MAPK) pathway together with phosphothreonine and phosphotyrosine residues present in STAT proteins [77]. In longer term studies, it is indicated that Mϕ expressing $CD68^+$ together with leukocytes expressing $CD45^+$ but also T cells (denoted by $CD3^+$) could also infiltrate heart ventricles and the choroid plexus [79]. Historically, and since 2021 the blood brain barrier (BBB) in terms of how immune cells permeate across semi–permeable membranes is unclear [80].

Antibody Responses to Ebola Virus

In the early 21st century between 2008–2009 reports appeared utilising an isolated neutralising antibody binding to EBOV GP to elucidate crystalline structure to an antibody [81]. It was then specified as *in vivo* research continued that a mouse Ig2a monoclonal antibody could bind specifically to the glycosylated mucin-like domain of the EBOV GP (EQHHRRTDN, amino acids 405–413) [82]. The overall structure of EBOV GP seemingly then was considered to be a GP1 trimer composed of base, head and glycan cap forming a chalice with a trimer of GP2 sub–units forming a cradle around the GP1 domain when investigations began to indicate differential antibody responses against GP1/GP2 and the sGP cellular product [83]. Furthermore the existence of a smaller Δ–peptide clarity remains unknown to date with two theories which are that of a peptide that could be immuno–adhesive or act as a viroporin [84–87]. To this effect during 2011, observations were made in comparison with different *Filoviridae* between sGP and ssGP function with hints of differences in the immunological function of the Δ–peptide resulting from a C–terminal cleavage product of sGP [87]. However longitudinal research studies of the 2013–2016 outbreak, indications were that a poly–functional B cell and APC induced $CD8^+$ T cell phenotype remained crucial (n=206) for an effective immune response. This was measured as cells being able to synthesise and secrete type II IFN–γ alongside two other cytokines being TNF and IL–2 synthesised in 0.046% of total $CD8^+$ T cells [88]. Observations of immunological relevance were examined within a cohort (n=207), where 96% possessed antibodies after EBOV infection with sub–characterisation confirming that 9% of blood samples were considered to possess neutralising antibodies (nAb) [88]. It was observed in Western Blot analysis that antibodies could be poly–functional against four of the seven EBOV virion encoded proteins (GP, NP, VP35 and VP40). Therefore, this would indicate characterised viral specific epitopes within EBOV particles that may generate antigen/epitope specific innate and/or adaptive immunological responses [88]. In longer term studies utilising development of a cell–based reporter system the affinity of EBOV stimulated antibody binding via their respective Fc (constant) receptors to effector cell surface receptors was examined. It was confirmed that EBOV infection may generate IgG1 antibodies that display the highest affinity for the FcγR1 (CD64) receptor rather than FcγRIII (CD16) and FcγRII (CD32) over a time–frame of more than 10 years [89]. These three receptors are utilised differentially by antibody Fc regions to effect B cell antibody dependant responses on a variety of host immune cells like macrophages.

Serology reports in 2020 are indicative that EBOV protective antibodies can be synthesised in humans after infection with iso–types produced predominantly ten times higher concentration of the IgG1 iso–type compared to IgG2, IgG3 and IgG4 still produced [90]. In addition, IgM is also produced that is historically seen to be a marker of natural infection in other viral pathologies. It is further indicated (n=4) in a longer kinetics study that IgG specific for GP remained over 2 years and up to 12

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years with IgM declining and IgA remaining at increased concentrations alongside IgG responses to both EBOV NP and VP40 [91]. The characterisation of B cells producing antibodies then was indicative of antibody secreting B cells (ASC) expressing $CD27^{hi}/CD38^{hi}$ with less expression of other activation markers $CD71^{+}/CD20^{+}$. Notable at this point was that removal of mucin and glycan caps of EBOV GP further increased binding affinity of serum IgG to cleaved viral GP (sGP) [92].

Adaptive Immune Responses to Ebola Virus

A key report utilised a control group of non–pathogenic *Filoviridae* (REBOV) in comparison with human pathogenic *Filoviridae* (EBOV, MARV) in both humans and macaques performed in 2006 [66]. It was noted, utilising annexin V and propidium iodide staining, that T cell apoptotic rates at least doubled indicating cell cycle changes and apoptotic cells, but not in the non–pathogenic control virus group (REBOV). Furthermore, utilising *in vitro* viral peptide sequences stimulating PBMCs and measuring hypodiploid DNA content that a three–fold DNA synthesis reduction occurring during the T cell synthesis (S) phase to each of the pathogenic *Filoviridae* (MARV/EBOV) was apparent [66]. It was considered that pathogenic *Filoviriade* could alter CD4/C8 cell count ratios measured by T_H1, T_H2 and T_C cell populations with reasons unknown as to cellular mechanisms. Indications may be suggestive that this could be caused by nuclear membrane changes, cell communication, intra–cellular signaling, or single nucleotide point mutations (SNP) that affect any of the proteins or immune cell populations. However EBOV T cell research is evolving and continues with T cell sub–types only recently researched that include recent mucosal associated invariant T cells (MAIT) and others [32].

It was noted in 2003 that EBOV may suppress T_H1 cells alongside IL–12p40 and type II IFN–γ production with inhibition of IL–2, TNF–α, CCL2 but not CCL3 utilising monoclonal antibody reagents that are validated as specific in research [60]. The reasons for this remain unclear. Further indications evidenced then were reduction in CD25 within T cell populations categorised into regulatory T cell (T_{REG}) amongst others known to express this soluble secreted PM cellular receptor . Shortly after in 2011, independently, T cell populations were further researched and it was noted that a memory T cell subset could possess regenerative–like properties (denoted by $CD45RO^{-}CCR7^{+}CD45RA^{+}CD62L^{+}CD27^{+}CD28^{+}$) and IL–$7R^{+}$ T cell compartment characteristic of T cells [93]. Importantly it indicated that T cells could be specific for viral and tumour associated antigens (TAA) whilst specifically expressing high concentrations of IL–2Rβ, CXCR3, and LFA–1 with the CD95 activation marker [93]. The role of T cell responses to EBOV in 2015 indicated that poly–functional T_C ($CD8^{+}$) cells could express CCR7 and not CD45RO as phenotypical T_{EM} cells developing EBOV specific responses upregulating CD28 and CD95 in response [40,94]. These were critical as CCR7 is expressed on the majority of the T cell population whereas CD45RO/RA is used to define T cell maturation while CD28 is a required co–stimulatory molecule required for T_C cell stimulation . Other investigations of the exact mechanism of action of EBOV on T cells further are indicative of a "superagonist–like" effect. When EBOV stimulated PBMCs were depleted of DCs/monocytes it could be seen that activation markers CD25/CD69 were significantly upregulated at 48 hours on T cells [40]. The reasons for EBOV stimulation and effects on host cells remain unknown. Below is shown an infographic of the overall immune system interactions based on prior research (see Figure 4).

With regards to the $CD4^{+}$ T cell population, stimulation indicated preferential expression of $CD45RO^{+}$ on T cells in survivors predominantly central memory (T_{CM}) T cells expressing $CXCR3^{+}$ that were EBOV GP antigen–specific with the overall T_H ($CD4^{hi}$) cell population expressing another activation (CD38) marker. Simultaneously in a 2018 report it was noted *in vivo* that CD69 expression downregulation did not occur within myeloid derived DCs (mDCs) in the spleen [95]. Though the earlier report did indicate that $CD4^{+}$ and $CD8^{+}$ T cells significantly downregulated CD69 at 12 hours, it was recently confirmed that this occurred at 48 hours with CD25 upregulation in T cells [66]. During 2019, annexin–A5 was suggested to potentially be a beneficial therapeutic during micro–vascular disease. Annexin proteins are both soluble and hydrophilic and bind to oppositely charge phospholipids reversibly dependent on calcium. Such research would be interesting to see as above

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annexin proteins can aggregate vesicles but also phosphatidylserine (PS) does appear to be implicated in EBOV host cell interaction mostly unknown [96,97].

Notable further investigations into EBOV host cell receptors showed that deficiency of TIM–1 *in vitro* cell line culture could regulate T cell secretion of crucial cytokines that were type II IFN–γ, IL–2, and TNF–α as well as IL–12p40 with the granulocyte-macrophage colony-stimulating factor (GM–CSF) [40]. Separately research evidenced EBOV stimulated expansion of $CD3^{lo}CD4^{hi}$ cells in comparison with a superantigen *Staphylococcal enterotoxin B* (SEB). Much remains unknown regarding EBOV effect on T cells; however it was evidenced that EBOV could both stimulate T cells and be internalised without replicating. Investigations showed the EBOV NP protein could be found within T cells expressing the TCR complex differentially affected. Specifically, the TCR CD3ε sub–unit domain could be found at increased frequency in EBOV stimulated samples. This was accompanied by downregulation and degradation of the TCR CD3ζ with intra–cellular signaling maintained but CD3ζ co–localised with a monomeric guanosine–5'–triphosphate (GTP) enzyme (a GTPase, Rab7) and EBOV GP in late endosomes [98–100]. The CD3/TCR complex predominantly utilises lymphocyte–specific protein tyrosine kinase (Lck) with phosphorylation of the TIM–1 cytoplasmic domain resulting in activating phosphatidylinositol 3–kinase (PI3K) representing a known cell survival and proliferation pathway. In other longitudinal kinetic studies (n=2), individuals underwent treatment, the CD95 marker was observed as transiently upregulated on both T_C/T_H cells with PD–1 transiently upregulated on T_H cells indicative of cellular activation. Antigen presentation peptides denoted by HLA-DR (MHC Class II) were present on $CD8^+$ T cell PMs but it was inferred that CD45RA and CCR7 may not be expressed on all T cell populations [101]. During EBOV infection, each of the DCs, monocytes, Mϕ and T cell branches of the immune system are predominantly affected similarly to other viral pathologies [32,102]. Recent observations are that EBOV sequesters the interferon regulatory factor (IRF3) in viral inclusion bodies (VIB) blocking required type I IFN induction. This usually occurs through IRF3 binding to the TNFR associated factor (TRAF) associated proteins usually activating a NF-κB kinase (TANK/TBK1) and the IκB kinase epsilon (IKKε) [103,104]. It is plausible that some of the kinetic studies outlined above that many of the delayed onset of immune cell upregulation of CD molecules could be regulated by type I/II/III IFN unknown so far; because in conjunction with the tolerogenic profile of DCs that T and NK cell responses usually stimulated may be seemingly affected by EBOV sub–unit protein domains that are toxic with the appearance of agonist like effects . However, the cytokine IL–8 (CXCL8) is known to differentially affect $CD4^{+/-}$ T cell activation of effector memory (T_{EM}) $CD4^{+/-}$ T cells but not naive (T_N) or central memory (T_{CM}) $CD4^{+/-}$ T cells. In addition, IL–8 can up-regulate IL–2 with no effect on type II IFN–γ and IL–4 production [105]. These observations remain key as IL–8 effect on T cell apoptosis remains unclear.

Natural Killer Cells during Immune Responses to Ebola Virus

During 2017 authors indicated using digital cell quantification (DCQ) that NK cells showed an evidential increase after infection during recovery from EBOV disease. In a comparison (n=112) transcriptome analysis with non– human primates (NHP) clear significance was noted between non–fatal and fatal outcomes of pro–inflammatory chemokines mRNA gene transcripts (*CXCL10, CCL2, CCL8, CXCL11*) during severe EBOV infection of which two have affinity to a known receptor present on DCs that is CXCR3 [106]. Shortly after in 2019, laboratory research investigated the role of Mϕ and NK cells (sCD163) in comparison given the similarities that severe EBOV disease has led to Mϕ activation syndrome (MAS) and haemophagocytic lymphohistiocytosis (HLH). At this juncture it was hypothesised that three cytokines (IL–6, IL–8, M–CSF), three chemokines (CCL2, CCL3 and CCL4), alongside two modulatory cytokines IL–10/IL–1RA remained pertinent to immunological cell function and migration during EBOV infection [72]. The T cell marker expressed by NK cells denoted as an immune cell shed soluble receptor (sIL–2R) present on T_{REG} ($CD25^+$) cells was also found at increased levels during infection [72] . As above, *in vivo*, TLR4 was considered to be an activation marker instigated by both pathogenic / non–pathogenic viruses. Authors noted that overall NK cell frequencies may be considered slightly reduced reflecting unknown NK cell activation and proliferation then. Furthermore a decrease in $CD56^{dim/bright}$ NK cells, alongside increased frequencies

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of CD56^{neg} NK cells hold possibilities and may be attributed as positive regulators of immunological relevance unknown to date [72].

More recently during 2020, the role that NK cells perform indicated that CD14^{+} monocytes may be considered the source of pro–inflammatory cytokines. *In vitro* stimulation/inhibition studies using EBOV GP indicated that NK cell activation was dependant on IL–18 and IL–12 and could be enhanced by an antagonist that blocked the IL–10 receptor [107]. Two NK cell activation markers, CD107a and CD25, were observed to be upregulated in response to EBOV GP but without secretion of type II IFN–γ usually synthesised by both NK and T cells [108]. Simultaneously the EBOV VP40 protein sub–unit domain was found to stimulate IL–12/IL–18 production [109]. But it was determined that the degranulation and cytolytic actions of NK cells were dependant on IL–12 to activate CD56^{+} NK cells. These observations noted that NK cell function and maturation was crucial. Interleukin–18 is considered to act in concert with IL–12 as a regulator of T_H1 differentiation along with IL–23 and regulates T_H17 secretion of IL–17. Dual regulatory effects can occur with activation of T_C cells and NK cells [110]. Activation markers like CD95 are used to examine NK cell maturation and observed to be upregulated without change during EBOV infection; but concurrently more recently sub–types of immune cells have been characterised that include CCR7^{neg} effector Vδ2 cells that can be an antigen–stimulated source of TNF–α [111]. The other activation marker, CD69, was expressed on each of the observed NK sub–types. As above type II IFN can inhibit EBOV infection and be produced by the above four cell types. Some of the unknown genetic mechanisms of type II IFN synthesis became slightly clearer with predominant ISG gene transcripts researched and observed that are relevant. Initially guanylate binding protein 5 (*GBP5*) upregulation was observed that is a factor that can inhibit type II IFN in other viral infections like Respiratory Syncytial virus (RSV) [112] ; but also others were observed that may affect other immune cell types regulating retinoic acid receptor responder protein 3 (*RARRES3*) and vesicle–associated membrane protein (*VAMP5)* as yet unknown [70,113].

Very recently, developments occurring showed the neutrophil chemoattractant IL–8 (CXCL–8) together with serum IL–1RA, TNF–α, CCL2 and TGF–α as essential factors in beneficial immune responses [54]. Metabolic enzymes were depicted as significant including high–density lipoproteins (HDL) that were apolipoproteins (E/L1/A4/C3/C4). Notably CSF1, GM–CSF, CCL3, CX3CL1, and a binding protein to IL–18 were associated with high EBOV viral loads but VP40 was not associated in severity with GP associated with PCR cycle threshold (*CT)* values over 50 [54]. These reports in combination indicate that M–CSF is a key cytokine required during Mϕ development; but also IL–18 can independently mediate type II IFN–γ secretion from helper T cells (T_H1) (through IL–18 binding protein (BP)) and is usually produced by Mϕ that NK cells require for cytolytic function. The role of IL–18 BP in regulating IFN–γ during EBOV infection is worthy of exploring as it is produced by mononuclear cells but also IL–18 does signal between IL–1 receptor-associated kinases (IRAK1/4) and could plausibly affect T cell and NK cell responses [114,115]. The synergism of IL-18 activity with other cytokines does stimulate NK cell proliferation and cytotoxicity via production of perforins and granzyme B remaining unclear to date [116]. The duality of NK cell function remains unclear as a multitude of activation and inhibitory receptors exist regulating NK cell dependant cytotoxicity; however activation/inhibition signaling was further researched in 2022. Researchers Jarahian et al. (Heidelberg, Germany) performed *in vitro* research to observe the EBOV GP may stimulate NK cell cytotoxic activation receptors (NKp44/NKp46) as well as selectin adhesion receptors (CD62L/CD62P) and inhibitory sialylated Siglec–7 (CD328) as well as Siglec–5 (CD170) receptor proteins [28]. These were significant findings because CD62L is constitutively expressed and required by leukocytes for cellular migration across EC layers, with CD62P similarly on platelets; while short chain CD328 (also CD170) contains immuno–receptor tyrosine–based inhibitory motif (ITIM) cellular cytoplasmic domains and external domain receptors which bind to glycans containing sialic acid.

Recent developments during *in vivo* transcriptional research demonstrate that during EBOV infection (n=9) that four genes are markedly upregulated at all stages of infection (*IRF7*, *S100A8*, *S100A9*, *IFI44*). These correspond to calprotectin and IRF proteins potentially encoded [117]. A further seven mRNA transcripts were noted at early stages of infection (*CD19, FCGR3, PRDM1, IRF1, CXCR2, IL13RA1, CD79A*) which do encode proteins that all may

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regulate B cells, antibody receptors, cytokines, receptors and chemokine receptor synthesis [117]. Importantly one key microRNA (miRNA) regulating post–transcriptional gene expression was highlighted and is under investigation in sepsis research. Future research on miR–122–5p will be very interesting to see. Recently, miR–122–5p was observed to regulate coagulation where mimics of miR–122–5p (n=84) were observed to downregulate IL–1β, IL–6, CCL2, and TNF–α in sepsis models [118]. Therefore these observations may have direct relevance to research in the future given that miRNA modulation of cytokine levels could potentially be applicable to different pathologies after extensive further research. More recently nine EBOV GP epitopes were examined against HLA alleles *in silico* to predict that thirteen HLA–A and HLA–B alleles may represent optimal $CD8^+$ responses required to generate broad immunological responses with two predicted to be within regions of the GP1.1 and GP2.2 domains [119]. Below is shown interpretation of current immunological response factors so far based on research to date (see Figure 5)

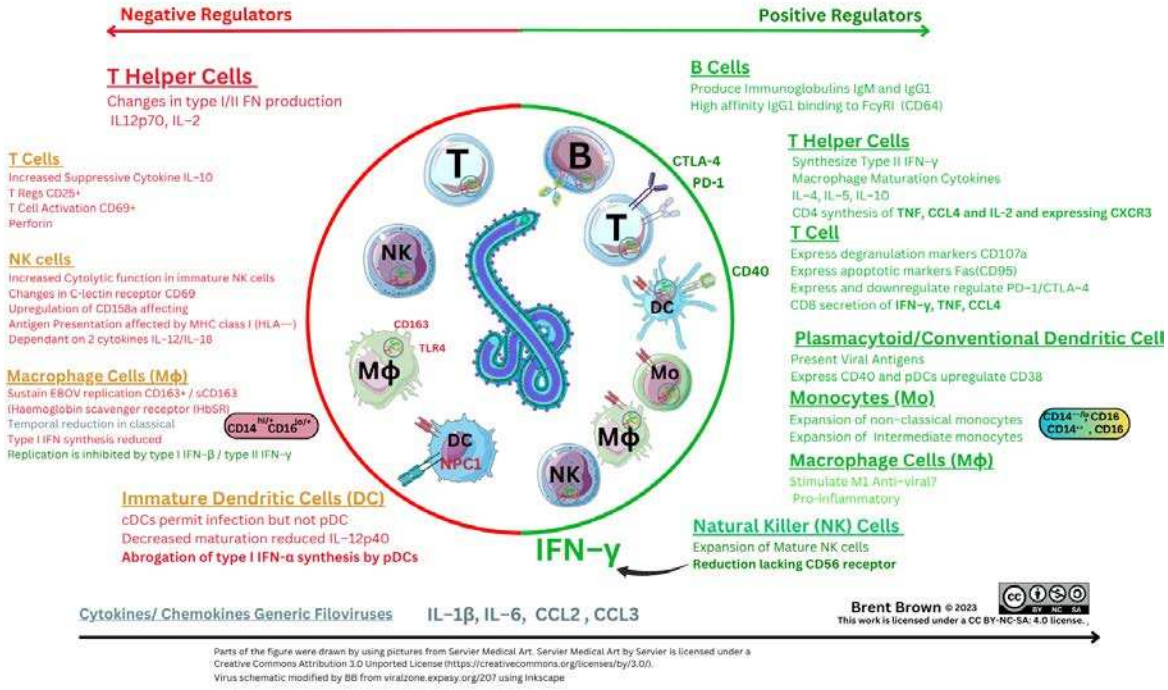

Figure 5. Overall Immunological Factors during Ebola Virus Infection.

Limitations

Vaccine and clinical trial studies are documented in other reviews [120]. In 2014, limitations of polymerase chain reaction (PCR) testing were noted with a report outlining potential options of lateral flow immunoassays (LFI) as a potential accurate viral antigen diagnostic [6,7,121]. Other articles discuss monoclonal antibodies that have been identified and historically are undergoing further research as both diagnostic and therapeutic antibodies are expensive and require validation for sensitivity and specificity which can vary between proteins in different host species [122,123]. Furthermore, single nucleotide point mutations (SNP) can occur in any cellular host gene encoding any protein [124]. However much remains unclear with research ongoing.

Discussion

During individual host immune response to pathogens, overall host innate and adaptive immune response may remain consistent as viral genomes will differ upon transcription and translation between species. As a prerequisite, if it is considered that both DNA and RNA viruses evolve and mutate, with more or less severity regardless, host immune proteins can vary with errors that occur during gene transcription and translation into proteins. Previously we discussed the $CD4^+$

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T cell upregulation during EBOV infection. This is important in determining the T–cell response and contribution to disease phenotype. Therapeutic strategies used may vary and the differential role of TIM–1 in T cell signaling is still being clarified [125]. Recent suggestions are that EBOV is dependent on phosphosphatidylserine with caspase dependant scramblases (XK–related protein (Xkr)) utilised further elucidating EBOV intracellular pathways [126]. The role of STAT protein mutations, and chemokines are still being discovered; but also type II IFN–γ was discovered some 50 years ago with errors and remains unknown within populations globally. Historically, errors in type II IFN–γ signaling are rarely documented in research or literature in mycobacterial infection and remains a key determinant of adaptive T cell function in pathogenic host immune cell responses [127,128]. Interferon research began in 1965 with the discovery of type I IFNs with the key role of type II IFN–γ in T cell anti–viral responses shortly after. More recently, 2003 discovery of type III IFN–λ and four subtypes remains largely unknown as to cellular signaling and transduction mechanisms. It can be seen in the diagrams above that there is an outline as to overall EBOV host immune responses. However chemokines were also only characterised between 2000–2014, to highlight atypical chemokine receptors (ACKRs) present within epithelial layers. In addition TLR research during EBOV infection is comparatively recent [124]. During any infection caused by viral, bacterial or mycobacterial pathogens, overall risk is usually defined by R0 as the transmission rate. This is a measure denoting pathogen transmission referred to as the reproduction number or how many people could be affected during contagious infectious diseases. It is reported that in 2015 that this was estimated for EBOV to be in the range of 1.37–2.53. Other reports indicate that overall risk of EBOV infection estimate was around 45% estimated within community settings [129,130]. The infection fatality rate (IFR) of EBOV between male/females is indicated with slight increased survival rate of EBOV affected females [131]. Given EBOV severity in disease it is prudent to consider that adaptive immune systems can vary by age with thymic T cell development mainly documented during and after cytomegalovirus (CMV) infection [132–134].

In the 21st century as real–time polymerase chain reaction (PCR) has become regularly used for sequencing viral genomes; Ebola virus has been investigated with regards to effects in different age–ranges. It was seen that a characterised marker, regulated upon activated T cell expressed and secreted molecule known as RANTES (CCL5) appears to correlate with survival outcome but also that two soluble adhesion molecules (sICAM, sVCAM) and plasminogen activator inhibitor–1 (PAI–1) released by endothelial cells were detrimental factors. These can vary in different age ranges and cellular mechanisms will require further clinical and laboratory research [135]. Also of note given the complexities of EBOV infection and disease together with the immune system, there are immunological characteristics that appear consistent between studies. As recently as 2021, other authors agree that the complexities of IL–10 regulation of innate lymphoid cells including NK cells will require clarity [136]. Furthermore, the role that pDCs play in immune systems similarly requires clarity as to mechanisms of IFN regulation in a cell population where cell viability and culture provides difficulty.

Further research on maturation between DCs, monocytes and macrophages will be required but also the mechanisms that Ebola virus proteins like VP24 may use to regulate CD38 and type I/III IFN synthesis are as yet unknown[52]. Questions remain given other recent research with similarities as outlined above with macrophage activation syndrome, but also sepsis appearing similar within systemic conditions of such severity. These observations will undoubtedly require further research in the future. Uniquely, a group in Seattle, USA, (Bruchez et al.) recently characterised a transcription factor (the major histocompatibility complex class II transactivator) that seemingly has 2–3 times proprietary anti–viral activity against two viruses (both Ebola as well as SARS–CoV–2) and is induced by type II interferon whilst inducing resistance through a CD74/p41 isoform antagonising a known host cathepsin L protease required to allow endocytosis of viral proteins [137]. However given the above, further research is required on the other immunological relevant antibodies that include sub–types of IgG (1–4), IgA (1–2) alongside relevant cellular function of natural killer cells, mast cells, basophils and eosinophils. Therapeutic strategies that are under research investigation for *Filoviridae* (EBOV/MARV) include anti–virals, polyclonal/monoclonal antibodies as well as

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proprietary small–interfering RNA (siRNA) molecules. It is currently indicated that there are at least 115 clinical trials at various stages globally on the National Institute of Health (NIH) website either completed or in progress. Of these three vaccines are at various stages of development that are viral–vector modified with two monoclonal antibody therapeutics recently recommended by the WHO (see Supplementary Materials) [120,138–140].

Conclusions

In conclusion, the immunological systemic innate and adaptive responses remain under–researched in many viral and pathological fields, and this is particularly true for Ebola virus. The factors we have outlined are immunological characteristics of cellular basis. Positive indicators of immunological relevance are upregulation of T helper ($CD4^+$) cell alongside the cytotoxic T ($CD8^+$) cell response as illustrated in Figure 4. The T cell phenotype will remain crucial to elucidate the dynamics of Ebola virus immune responses and other pathologies as well in future. The role of pattern recognition receptors including Toll–like receptors and other immune cell types like mucosal associated invariant T cells will also require further investigation as will other receptors relevant to T cell signaling responses. There are currently 371 CD molecules classified that immune cells could express with variable cellular function. Additionally there are 10 Toll-like Receptors classified within the CD nomenclature that remain unclear as to relevance within how or why Ebola virus evokes such differential immunological characteristics. Although technological advancements and transcriptome studies allow further clarity and insights into mechanisms underlying scientific progress; Human leukocytes alleles may also vary between global populations encoding major histocompatibility complexes (type I/II) required to present viral peptide antigens to systemic immune cells.

Characterisation of the Ebola virus genome is ongoing, and combined with technological advancements and transcriptome studies will further advance clarity and insights into mechanisms. A holistic overview is of utility, however, for which we have provided known research to date. The authors therefore hope that the comprehensive information presented will be useful for clinicians, academics and researchers alike into the future.

Supplementary Materials: Figure 1: Ebola Virus Genome**; Figure 2**: Ebola Virus Cellular Pathways; **Figure 3:** Ebola Virus Pathogenesis **Table 1**: Ebola Virus Proteins and Gene Regulation Factors**; Table 2**: Cytokine Regulators during Ebola Virus Infection**; Figure 4**: Immune Cellular Response Profile by Cluster of Differentiation (CD) molecule during EBOV infection; **Figure 5**: Overall Immunological Factors during Ebola Virus Infection; Predictive values of Ebola RDTs and implications for decision-makers; Ebola Best Practice; Search Results | Beta ClinicalTrials.gov; WHO makes new recommendations for Ebola treatments, calls for improved access

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List of abbreviations: Lymphocyte function–associated antigen 1 (LFA–1), Intercellular Adhesion Molecule (ICAM), Vascular Cell Adhesion Molecule (VCAM). For other protein abbreviations not listed see www.reactome.org

References

1. Barbiero VK. Ebola: A Hyperinflated Emergency. *Glob Health Sci Pract*. 2020;8(2):178-182. doi:10.9745/GHSP-D-19-00422
2. Althaus CL. Estimating the Reproduction Number of Ebola Virus (EBOV) During the 2014 Outbreak in West Africa. *PLoS Curr*. Published online 2014. doi:10.1371/currents.outbreaks.91afb5e0f279e7f29e7056095255b288
3. James SL, Abate D, Abate KH, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*. 2018;392(10159):1789-1858. doi:10.1016/S0140-6736(18)32279-7
4. Kallio-Kokko H, Uzcategui N, Vapalahti O, Vaheri A. Viral zoonoses in Europe. *FEMS Microbiol Rev*. 2005;29(5):1051-1077. doi:10.1016/j.femsre.2005.04.012
5. Adams MJ, Lefkowitz EJ, King AMQ, et al. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). *Arch Virol*. 2017;162(8):2505-2538. doi:10.1007/s00705-017-3358-5
6. Bettini A, Lapa D, Garbuglia AR. Diagnostics of Ebola virus. *Front Public Health*. 2023;11. doi:10.3389/fpubh.2023.1123024
7. Land KJ, Boeras DI, Chen XS, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nat Microbiol*. 2018;4(1):46-54. doi:10.1038/s41564-018-0295-3
8. Jain S, Martynova E, Rizvanov A, Khaiboullina S, Baranwal M. Structural and Functional Aspects of Ebola Virus Proteins. *Pathogens*. 2021;10(10):1330. doi:10.3390/pathogens10101330
9. Vetter P, Fischer WA, Schibler M, Jacobs M, Bausch DG, Kaiser L. Ebola Virus Shedding and Transmission: Review of Current Evidence. *Journal of Infectious Diseases*. 2016;214(suppl 3):S177-S184. doi:10.1093/infdis/jiw254
10. Sanchez A, Yang ZY, Xu L, Nabel GJ, Crews T, Peters CJ. Biochemical Analysis of the Secreted and Virion Glycoproteins of Ebola Virus. *J Virol*. 1998;72(8):6442-6447. doi:10.1128/JVI.72.8.6442-6447.1998
11. Yuan B, Peng Q, Cheng J, et al. Structure of the Ebola virus polymerase complex. *Nature*. 2022;610(7931):394-401. doi:10.1038/s41586-022-05271-2
12. Mehedi M, Falzarano D, Seebach J, et al. A New Ebola Virus Nonstructural Glycoprotein Expressed through RNA Editing. *J Virol*. 2011;85(11):5406-5414. doi:10.1128/JVI.02190-10
13. Lim JP, Gleeson PA. Macropinocytosis: an endocytic pathway for internalising large gulps. *Immunol Cell Biol*. 2011;89(8):836-843. doi:10.1038/icb.2011.20
14. Nanbo A, Imai M, Watanabe S, et al. Ebolavirus Is Internalized into Host Cells via Macropinocytosis in a Viral Glycoprotein-Dependent Manner. *PLoS Pathog*. 2010;6(9):e1001121. doi:10.1371/journal.ppat.1001121
15. Di Palma F, Daino GL, Ramaswamy VK, et al. Relevance of Ebola virus VP35 homo-dimerization on the type I interferon cascade inhibition. *Antivir Chem Chemother*. 2019;27:204020661988922. doi:10.1177/2040206619889220
16. Yu DS, Weng TH, Wu XX, et al. The lifecycle of the Ebola virus in host cells. *Oncotarget*. 2017;8(33):55750-55759. doi:10.18632/oncotarget.18498
17. Di Paola N, Sanchez-Lockhart M, Zeng X, Kuhn JH, Palacios G. Viral genomics in Ebola virus research. *Nat Rev Microbiol*. 2020;18(7):365-378. doi:10.1038/s41579-020-0354-7
18. Vidal S, Sánchez-Aparicio M, Seoane R, et al. Expression of the Ebola Virus VP24 Protein Compromises the Integrity of the Nuclear Envelope and Induces a Laminopathy-Like Cellular Phenotype. *mBio*. 2021;12(4). doi:10.1128/mBio.00972-21
19. Simon T, Bromberg JS. Regulation of the Immune System by Laminins. *Trends Immunol*. 2017;38(11):858-871. doi:10.1016/j.it.2017.06.002
20. Dubik N, Mai S. Lamin A/C: Function in Normal and Tumor Cells. *Cancers (Basel)*. 2020;12(12):3688. doi:10.3390/cancers12123688
21. Fels JM, Bortz RH, Alkutkar T, et al. A Glycoprotein Mutation That Emerged during the 2013–2016 Ebola Virus Epidemic Alters Proteolysis and Accelerates Membrane Fusion. *mBio*. 2021;12(1). doi:10.1128/mBio.03616-20

22. Li X, Wang J, Coutavas E, Shi H, Hao Q, Blobel G. Structure of human Niemann–Pick C1 protein. *Proceedings of the National Academy of Sciences*. 2016;113(29):8212-8217. doi:10.1073/pnas.1607795113
23. Martin B, Hoenen T, Canard B, Decroly E. Filovirus proteins for antiviral drug discovery: A structure/function analysis of surface glycoproteins and virus entry. *Antiviral Res*. 2016;135:1-14. doi:10.1016/j.antiviral.2016.09.001
24. Winter SL, Golani G, Lolicato F, et al. The Ebola virus VP40 matrix layer undergoes endosomal disassembly essential for membrane fusion. *EMBO J*. 2023;42(11). doi:10.15252/embj.2023113578
25. Soilleux EJ, Barten R, Trowsdale J. Cutting Edge: DC-SIGN; a Related Gene, DC-SIGNR; and CD23 Form a Cluster on 19p13 . *The Journal of Immunology*. 2000;165(6):2937-2942. doi:10.4049/jimmunol.165.6.2937
26. Tailleux L, Schwartz O, Herrmann JL, et al. DC-SIGN Is the Major Mycobacterium tuberculosis Receptor on Human Dendritic Cells. *Journal of Experimental Medicine*. 2003;197(1):121-127. doi:10.1084/jem.20021468
27. Bashirova AA, Geijtenbeek TBH, van Duijnhoven GCF, et al. A Dendritic Cell–Specific Intercellular Adhesion Molecule 3–Grabbing Nonintegrin (Dc-Sign)–Related Protein Is Highly Expressed on Human Liver Sinusoidal Endothelial Cells and Promotes HIV-1 Infection. *Journal of Experimental Medicine*. 2001;193(6):671-678. doi:10.1084/jem.193.6.671
28. Jarahian M, Marstaller K, Banna N, et al. Activating Natural Killer Cell Receptors, Selectins, and Inhibitory Siglecs Recognize Ebolavirus Glycoprotein. *J Innate Immun*. 2022;14(2):135-147. doi:10.1159/000517628
29. Mohamadzadeh M, Coberley SS, Olinger GG, et al. Activation of Triggering Receptor Expressed on Myeloid Cells-1 on Human Neutrophils by Marburg and Ebola Viruses. *J Virol*. 2006;80(14):7235-7244. doi:10.1128/JVI.00543-06
30. Herrero-Cervera A, Soehnlein O, Kenne E. Neutrophils in chronic inflammatory diseases. *Cell Mol Immunol*. 2022;19(2):177-191. doi:10.1038/s41423-021-00832-3
31. Hirano T. IL-6 in inflammation, autoimmunity and cancer. *Int Immunol*. 2021;33(3):127-148. doi:10.1093/intimm/dxaa078
32. Brown B, Ojha V, Fricke I, et al. Innate and Adaptive Immunity during SARS-CoV-2 Infection: Biomolecular Cellular Markers and Mechanisms. *Vaccines (Basel)*. 2023;11(2):408. doi:10.3390/vaccines11020408
33. Lanier LL. DAP10- and DAP12-associated receptors in innate immunity. *Immunol Rev*. 2009;227(1):150-160. doi:10.1111/j.1600-065X.2008.00720.x
34. Turnbull IR, Colonna M. Activating and inhibitory functions of DAP12. *Nat Rev Immunol*. 2007;7(2):155-161. doi:10.1038/nri2014
35. Wang H, Kadlecek TA, Au-Yeung BB, et al. ZAP-70: an essential kinase in T-cell signaling. *Cold Spring Harb Perspect Biol*. 2010;2(5). doi:10.1101/cshperspect.a002279
36. Mócsai A, Ruland J, Tybulewicz VLJ. The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nat Rev Immunol*. 2010;10(6):387-402. doi:10.1038/nri2765
37. Zhong L, Zhang ZL, Li X, et al. TREM2/DAP12 Complex Regulates Inflammatory Responses in Microglia via the JNK Signaling Pathway. *Front Aging Neurosci*. 2017;9. doi:10.3389/fnagi.2017.00204
38. Kondratowicz AS, Lennemann NJ, Sinn PL, et al. T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for *Zaire Ebolavirus* and *Lake Victoria Marburgvirus*. *Proceedings of the National Academy of Sciences*. 2011;108(20):8426-8431. doi:10.1073/pnas.1019030108
39. Hsu PL, Jou J, Tsai SJ. TYRO3: A potential therapeutic target in cancer. *Exp Biol Med*. 2019;244(2):83-99. doi:10.1177/1535370219828195
40. Younan P, Iampietro M, Nishida A, et al. Ebola Virus Binding to Tim-1 on T Lymphocytes Induces a Cytokine Storm. *mBio*. 2017;8(5). doi:10.1128/mBio.00845-17
41. Zhang Q, Yang J, Tillieux S, et al. Stepwise Enzymatic-Dependent Mechanism of Ebola Virus Binding to Cell Surface Receptors Monitored by AFM. *Nano Lett*. 2022;22(4):1641-1648. doi:10.1021/acs.nanolett.1c04677
42. Brunton B, Rogers K, Phillips EK, et al. TIM-1 serves as a receptor for Ebola virus in vivo, enhancing viremia and pathogenesis. *PLoS Negl Trop Dis*. 2019;13(6):e0006983. doi:10.1371/journal.pntd.0006983
43. Johnson KA, Taghon GJF, Scott JL, Stahelin R V. The Ebola Virus matrix protein, VP40, requires phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) for extensive oligomerization at the plasma membrane and viral egress. *Sci Rep*. 2016;6(1):19125. doi:10.1038/srep19125
44. Cioffi MD. Role of lipid composition on human plasma membrane interactions with the Ebola virus matrix protein VP40. *Biophys J*. 2023;122(3):506a-507a. doi:10.1016/j.bpj.2022.11.2698
45. Katan M, Cockcroft S. Phosphatidylinositol(4,5)bisphosphate: diverse functions at the plasma membrane. *Essays Biochem*. 2020;64(3):513-531. doi:10.1042/EBC20200041
46. Herbert AS, Davidson C, Kuehne AI, et al. Niemann-Pick C1 Is Essential for Ebolavirus Replication and Pathogenesis *In Vivo*. *mBio*. 2015;6(3). doi:10.1128/mBio.00565-15
47. Zhu Y, Shmidov Y, Harris EA, Theus MH, Bitton R, Matson JB. Activating hidden signals by mimicking cryptic sites in a synthetic extracellular matrix. *Nat Commun*. 2023;14(1):3635. doi:10.1038/s41467-023-39349-w

48. Fanunza E, Frau A, Corona A, Tramontano E. Insights into Ebola Virus VP35 and VP24 Interferon Inhibitory Functions and their Initial Exploitation as Drug Targets. *Infect Disord Drug Targets*. 2019;19(4):362-374. doi:10.2174/1871526519666181123145540
49. Coxon P, Rane M, Uriarte S, et al. MAPK-activated protein kinase-2 participates in p38 MAPK-dependent and ERK-dependent functions in human neutrophils. *Cell Signal*. 2003;15(11):993-1001. doi:10.1016/S0898-6568(03)00074-3
50. Zhou Y, Wu J, Liu C, et al. P38α has an important role in antigen cross-presentation by dendritic cells. *Cell Mol Immunol*. 2018;15(3):246-259. doi:10.1038/cmi.2016.49
51. Carroll SA, Towner JS, Sealy TK, et al. Molecular Evolution of Viruses of the Family Filoviridae Based on 97 Whole-Genome Sequences. *J Virol*. 2013;87(5):2608-2616. doi:10.1128/JVI.03118-12
52. Ilinykh PA, Lubaki NM, Widen SG, et al. Different Temporal Effects of Ebola Virus VP35 and VP24 Proteins on Global Gene Expression in Human Dendritic Cells. *J Virol*. 2015;89(15):7567-7583. doi:10.1128/JVI.00924-15
53. LaVergne SM, Sakabe S, Kanneh L, et al. Ebola-Specific CD8+ and CD4+ T-Cell Responses in Sierra Leonean Ebola Virus Survivors With or Without Post-Ebola Sequelae. *J Infect Dis*. 2020;222(9):1488-1497. doi:10.1093/infdis/jiaa268
54. Viodé A, Smolen KK, Fatou B, et al. Plasma Proteomic Analysis Distinguishes Severity Outcomes of Human Ebola Virus Disease. *mBio*. 2022;13(3). doi:10.1128/mbio.00567-22
55. Engel P, Boumsell L, Balderas R, et al. CD Nomenclature 2015: Human Leukocyte Differentiation Antigen Workshops as a Driving Force in Immunology. *The Journal of Immunology*. 2015;195(10):4555-4563. doi:10.4049/jimmunol.1502033
56. Xuan W, Qu Q, Zheng B, Xiong S, Fan GH. The chemotaxis of M1 and M2 macrophages is regulated by different chemokines. *J Leukoc Biol*. 2015;97(1):61-69. doi:10.1189/jlb.1A0314-170R
57. Sokol CL, Luster AD. The chemokine system in innate immunity. *Cold Spring Harb Perspect Biol*. 2015;7(5):a016303. doi:10.1101/cshperspect.a016303
58. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the Eye of the Cytokine Storm. *Microbiology and Molecular Biology Reviews*. 2012;76(1):16-32. doi:10.1128/MMBR.05015-11
59. Villani AC, Satija R, Reynolds G, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science (1979)*. 2017;356(6335). doi:10.1126/science.aah4573
60. Bosio CM, Aman MJ, Grogan C, et al. Ebola and Marburg Viruses Replicate in Monocyte-Derived Dendritic Cells without Inducing the Production of Cytokines and Full Maturation. *J Infect Dis*. 2003;188(11):1630-1638. doi:10.1086/379199
61. Wittling MC, Cahalan SR, Levenson EA, Rabin RL. Shared and Unique Features of Human Interferon-Beta and Interferon-Alpha Subtypes. *Front Immunol*. 2021;11. doi:10.3389/fimmu.2020.605673
62. Hammou RA, Kasmi Y, Khataby K, Laasri FE, Boughribil S, Ennaji MM. Roles of VP35, VP40 and VP24 Proteins of Ebola Virus in Pathogenic and Replication Mechanisms. In: *Ebola*. InTech; 2016. doi:10.5772/63830
63. Wiedemann A, Foucat E, Hocini H, et al. Long-lasting severe immune dysfunction in Ebola virus disease survivors. *Nat Commun*. 2020;11(1):3730. doi:10.1038/s41467-020-17489-7
64. Gessani S, Conti L, Del Cornò M, Belardelli F. Type I Interferons as Regulators of Human Antigen Presenting Cell Functions. *Toxins (Basel)*. 2014;6(6):1696-1723. doi:10.3390/toxins6061696
65. Martinez O, Johnson JC, Honko A, et al. Ebola Virus Exploits a Monocyte Differentiation Program To Promote Its Entry. *J Virol*. 2013;87(7):3801-3814. doi:10.1128/JVI.02695-12
66. Yaddanapudi K, Palacios G, Towner JS, et al. Implication of a retrovirus-like glycoprotein peptide in the immunopathogenesis of Ebola and Marburg viruses. *The FASEB Journal*. 2006;20(14):2519-2530. doi:10.1096/fj.06-6151com
67. Ullrich KAM, Schulze L Lou, Paap EM, Müller TM, Neurath MF, Zundler S. Immunology of IL-12: An update on functional activities and implications for disease. *EXCLI J*. 2020;19:1563-1589. doi:10.17179/excli2020-3104
68. Reizis B. Plasmacytoid Dendritic Cells: Development, Regulation, and Function. *Immunity*. 2019;50(1):37-50. doi:10.1016/j.immuni.2018.12.027
69. Falasca L, Agrati C, Petrosillo N, et al. Molecular mechanisms of Ebola virus pathogenesis: focus on cell death. *Cell Death Differ*. 2015;22(8):1250-1259. doi:10.1038/cdd.2015.67
70. Rhein BA, Powers LS, Rogers K, et al. Interferon-γ Inhibits Ebola Virus Infection. *PLoS Pathog*. 2015;11(11):e1005263. doi:10.1371/journal.ppat.1005263
71. Van Raemdonck K, Van den Steen PE, Liekens S, Van Damme J, Struyf S. CXCR3 ligands in disease and therapy. *Cytokine & Growth Factor Reviews*. 2015;26(3):311-327. doi:10.1016/j.cytogfr.2014.11.009
72. McElroy AK, Shrivastava-Ranjan P, Harmon JR, et al. Macrophage Activation Marker Soluble CD163 Associated with Fatal and Severe Ebola Virus Disease in Humans1. *Emerg Infect Dis*. 2019;25(2):290-298. doi:10.3201/eid2502.181326

73. Sharygin D, Koniaris LG, Wells C, Zimmers TA, Hamidi T. Role of <scp>CD14</scp> in human disease. *Immunology*. Published online March 27, 2023. doi:10.1111/imm.13634
74. Mass E, Nimmerjahn F, Kierdorf K, Schlitzer A. Tissue-specific macrophages: how they develop and choreograph tissue biology. *Nat Rev Immunol*. Published online March 15, 2023. doi:10.1038/s41577-023-00848-y
75. Weaver LK, Hintz-Goldstein KA, Pioli PA, et al. Pivotal Advance: Activation of cell surface Toll-like receptors causes shedding of the hemoglobin scavenger receptor CD163. *J Leukoc Biol*. 2006;80(1):26-35. doi:10.1189/jlb.1205756
76. Lai CY, Strange DP, Wong TAS, Lehrer AT, Verma S. Ebola Virus Glycoprotein Induces an Innate Immune Response In vivo via TLR4. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.01571
77. Hölzer M, Krähling V, Amman F, et al. Differential transcriptional responses to Ebola and Marburg virus infection in bat and human cells. *Sci Rep*. 2016;6(1):34589. doi:10.1038/srep34589
78. Purvis GSD, Solito E, Thiemermann C. Annexin-A1: Therapeutic Potential in Microvascular Disease. *Front Immunol*. 2019;10. doi:10.3389/fimmu.2019.00938
79. Liu J, Trefry JC, Babka AM, et al. Ebola virus persistence and disease recrudescence in the brains of antibody-treated nonhuman primate survivors. *Sci Transl Med*. 2022;14(631). doi:10.1126/scitranslmed.abi5229
80. Rustenhoven J, Drieu A, Mamuladze T, et al. Functional characterization of the dural sinuses as a neuroimmune interface. *Cell*. 2021;184(4):1000-1016.e27. doi:10.1016/j.cell.2020.12.040
81. Lee JE, Saphire EO. *Ebolavirus* glycoprotein structure and mechanism of entry. *Future Virol*. 2009;4(6):621-635. doi:10.2217/fvl.09.56
82. Lee JE, Kuehne A, Abelson DM, Fusco ML, Hart MK, Saphire EO. Complex of a Protective Antibody with Its Ebola Virus GP Peptide Epitope: Unusual Features of a Vλx Light Chain. *J Mol Biol*. 2008;375(1):202-216. doi:10.1016/j.jmb.2007.10.017
83. de La Vega MA, Wong G, Kobinger GP, Qiu X. The Multiple Roles of sGP in Ebola Pathogenesis. *Viral Immunol*. 2015;28(1):3-9. doi:10.1089/vim.2014.0068
84. Pokhrel R, Pavadai E, Gerstman BS, Chapagain PP. Membrane pore formation and ion selectivity of the Ebola virus delta peptide. *Physical Chemistry Chemical Physics*. 2019;21(10):5578-5585. doi:10.1039/C8CP07323F
85. He J, Melnik LI, Komin A, et al. Ebola Virus Delta Peptide Is a Viroporin. *J Virol*. 2017;91(16). doi:10.1128/JVI.00438-17
86. Melnik LI, Guha S, Ghimire J, et al. Ebola virus delta peptide is an enterotoxin. *Cell Rep*. 2022;38(1):110172. doi:10.1016/j.celrep.2021.110172
87. Radoshitzky SR, Warfield KL, Chi X, et al. Ebolavirus Δ-Peptide Immunoadhesins Inhibit Marburgvirus and Ebolavirus Cell Entry. *J Virol*. 2011;85(17):8502-8513. doi:10.1128/JVI.02600-10
88. Thom R, Tipton T, Strecker T, et al. Longitudinal antibody and T cell responses in Ebola virus disease survivors and contacts: an observational cohort study. *Lancet Infect Dis*. 2021;21(4):507-516. doi:10.1016/S1473-3099(20)30736-2
89. Radinsky O, Edri A, Brusilovsky M, et al. Sudan ebolavirus long recovered survivors produce GP-specific Abs that are of the IgG1 subclass and preferentially bind FcγRI. *Sci Rep*. 2017;7(1):6054. doi:10.1038/s41598-017-06226-8
90. Koch T, Rottstegge M, Ruibal P, et al. Ebola Virus Disease Survivors Show More Efficient Antibody Immunity than Vaccinees Despite Similar Levels of Circulating Immunoglobulins. *Viruses*. 2020;12(9):915. doi:10.3390/v12090915
91. Davis CW, Jackson KJL, McElroy AK, et al. Longitudinal Analysis of the Human B Cell Response to Ebola Virus Infection. *Cell*. 2019;177(6):1566-1582.e17. doi:10.1016/j.cell.2019.04.036
92. Lee JE, Fusco ML, Hessell AJ, Oswald WB, Burton DR, Saphire EO. Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. *Nature*. 2008;454(7201):177-182. doi:10.1038/nature07082
93. Gattinoni L, Lugli E, Ji Y, et al. A human memory T cell subset with stem cell–like properties. *Nat Med*. 2011;17(10):1290-1297. doi:10.1038/nm.2446
94. Tipton TRW, Hall Y, Bore JA, et al. Characterisation of the T-cell response to Ebola virus glycoprotein amongst survivors of the 2013–16 West Africa epidemic. *Nat Commun*. 2021;12(1):1153. doi:10.1038/s41467-021-21411-0
95. Lavender KJ, Williamson BN, Saturday G, et al. Pathogenicity of Ebola and Marburg Viruses Is Associated With Differential Activation of the Myeloid Compartment in Humanized Triple Knockout-Bone Marrow, Liver, and Thymus Mice. *J Infect Dis*. 2018;218(suppl_5):S409-S417. doi:10.1093/infdis/jiy269
96. Kennedy JR. Phosphatidylserine's role in Ebola's inflammatory cytokine storm and hemorrhagic consumptive coagulopathy and the therapeutic potential of annexin V. *Med Hypotheses*. 2020;135:109462. doi:10.1016/j.mehy.2019.109462
97. Bohan D, Van Ert H, Ruggio N, et al. Phosphatidylserine receptors enhance SARS-CoV-2 infection. *PLoS Pathog*. 2021;17(11):e1009743. doi:10.1371/journal.ppat.1009743

98. Érsek B, Molnár V, Balogh A, et al. CD3ζ-Chain Expression of Human T Lymphocytes Is Regulated by TNF via Src-like Adaptor Protein-Dependent Proteasomal Degradation. *The Journal of Immunology*. 2012;189(4):1602-1610. doi:10.4049/jimmunol.1102365
99. Bozso SJ, Kang JJH, Nagendran J. The role of competing mechanisms on Lck regulation. *Immunol Res*. 2020;68(5):289-295. doi:10.1007/s12026-020-09148-2
100. Bhuin T, Roy JK. Rab proteins: The key regulators of intracellular vesicle transport. *Exp Cell Res*. 2014;328(1):1-19. doi:10.1016/j.yexcr.2014.07.027
101. Agrati C, Castilletti C, Casetti R, et al. Longitudinal characterization of dysfunctional T cell-activation during human acute Ebola infection. *Cell Death Dis*. 2016;7(3):e2164-e2164. doi:10.1038/cddis.2016.55
102. Brown B, Gravier T, Fricke I, et al. Immunopathogenesis of Nipah Virus Infection and Associated Immune Responses. *Immuno*. 2023;3(2):160-181. doi:10.3390/immuno3020011
103. AL Hamrashdi M, Brady G. Regulation of IRF3 activation in human antiviral signaling pathways. *Biochem Pharmacol*. 2022;200:115026. doi:10.1016/j.bcp.2022.115026
104. Wu X, Qi X, Qu B, et al. Evasion of Antiviral Immunity through Sequestering of TBK1/IKKε/IRF3 into Viral Inclusion Bodies. *J Virol*. 2014;88(6):3067-3076. doi:10.1128/JVI.03510-13
105. Meniailo ME, Malashchenko VV, Shmarov VA, et al. Direct effects of interleukin-8 on growth and functional activity of T lymphocytes. *Int Immunopharmacol*. 2017;50:178-185. doi:10.1016/j.intimp.2017.06.023
106. Liu X, Speranza E, Muñoz-Fontela C, et al. Transcriptomic signatures differentiate survival from fatal outcomes in humans infected with Ebola virus. *Genome Biol*. 2017;18(1):4. doi:10.1186/s13059-016-1137-3
107. Wagstaffe HR, Clutterbuck EA, Bockstal V, et al. Ebola virus glycoprotein stimulates IL-18–dependent natural killer cell responses. *Journal of Clinical Investigation*. 2020;130(7):3936-3946. doi:10.1172/JCI132438
108. Wagstaffe HR, Anzala O, Kibuuka H, et al. NK Cell Subset Redistribution and Antibody Dependent Activation after Ebola Vaccination in Africans. *Vaccines (Basel)*. 2022;10(6):884. doi:10.3390/vaccines10060884
109. Le H, Spearman P, Waggoner SN, Singh K. Ebola virus protein VP40 stimulates IL-12– and IL-18–dependent activation of human natural killer cells. *JCI Insight*. 2022;7(16). doi:10.1172/jci.insight.158902
110. Esmailbeig M, Ghaderi A. Interleukin-18: a regulator of cancer and autoimmune diseases. *Eur Cytokine Netw*. 2017;28(4):127-140. doi:10.1684/ecn.2018.0401
111. Cimini E, Viola D, Cabeza-Cabrerizo M, et al. Different features of Vδ2 T and NK cells in fatal and non-fatal human Ebola infections. *PLoS Negl Trop Dis*. 2017;11(5):e0005645. doi:10.1371/journal.pntd.0005645
112. Li Z, Qu X, Liu X, et al. GBP5 Is an Interferon-Induced Inhibitor of Respiratory Syncytial Virus. *J Virol*. 2020;94(21). doi:10.1128/JVI.01407-20
113. Anderson AM, Kalimutho M, Harten S, Nanayakkara DM, Khanna KK, Ragan MA. The metastasis suppressor RARRES3 as an endogenous inhibitor of the immunoproteasome expression in breast cancer cells. *Sci Rep*. 2017;7(1):39873. doi:10.1038/srep39873
114. Zhou T, Damsky W, Weizman OE, et al. IL-18BP is a secreted immune checkpoint and barrier to IL-18 immunotherapy. *Nature*. 2020;583(7817):609-614. doi:10.1038/s41586-020-2422-6
115. Dinarello CA, Novick D, Kim S, Kaplanski G. Interleukin-18 and IL-18 Binding Protein. *Front Immunol*. 2013;4. doi:10.3389/fimmu.2013.00289
116. Choi YH, Lim EJ, Kim SW, Moon YW, Park KS, An HJ. IL-27 enhances IL-15/IL-18-mediated activation of human natural killer cells. *J Immunother Cancer*. 2019;7(1):168. doi:10.1186/s40425-019-0652-7
117. Stefan CP, Arnold CE, Shoemaker CJ, et al. Transcriptomic Analysis Reveals Host miRNAs Correlated with Immune Gene Dysregulation during Fatal Disease Progression in the Ebola Virus Cynomolgus Macaque Disease Model. *Microorganisms*. 2021;9(3):665. doi:10.3390/microorganisms9030665
118. Wang H, Zhang C, Zhang C, Wang Y, Zhai K, Tong Z. MicroRNA-122-5p regulates coagulation and inflammation through MASP1 and HO-1 genes. *Infection, Genetics and Evolution*. 2022;100:105268. doi:10.1016/j.meegid.2022.105268
119. Powlson J, Wright D, Zeltina A, et al. Characterization of Antigenic MHC-Class-I-Restricted T Cell Epitopes in the Glycoprotein of Ebolavirus. *Cell Rep*. 2019;29(9):2537-2545.e3. doi:10.1016/j.celrep.2019.10.105
120. Woolsey C, Geisbert TW. Current state of Ebola virus vaccines: A snapshot. *PLoS Pathog*. 2021;17(12):e1010078. doi:10.1371/journal.ppat.1010078
121. Wonderly B, Jones S, Gatton ML, et al. Comparative performance of four rapid Ebola antigen-detection lateral flow immunoassays during the 2014-2016 Ebola epidemic in West Africa. *PLoS One*. 2019;14(3):e0212113. doi:10.1371/journal.pone.0212113
122. Tshiani Mbaya O, Mukumbayi P, Mulangu S. Review: Insights on Current FDA-Approved Monoclonal Antibodies Against Ebola Virus Infection. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.721328
123. Yamaoka S, Ebihara H. Pathogenicity and Virulence of Ebolaviruses with Species- and Variant-specificity. *Virulence*. 2021;12(1):885-901. doi:10.1080/21505594.2021.1898169
124. Su S, Tao L, Deng Z, Chen W, Qin S, Jiang H. TLR10: Insights, controversies and potential utility as a therapeutic target. *Scand J Immunol*. 2021;93(4). doi:10.1111/sji.12988

125. Zhang M, Wang X, Hu L, et al. TIM-1 Augments Cellular Entry of Ebola Virus Species and Mutants, Which Is Blocked by Recombinant TIM-1 Protein. *Microbiol Spectr*. 2022;10(3). doi:10.1128/spectrum.02212-21
126. Nanbo A, Maruyama J, Imai M, et al. Ebola virus requires a host scramblase for externalization of phosphatidylserine on the surface of viral particles. *PLoS Pathog*. 2018;14(1):e1006848. doi:10.1371/journal.ppat.1006848
127. Ivashkiv LB. IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. *Nat Rev Immunol*. 2018;18(9):545-558. doi:10.1038/s41577-018-0029-z
128. Kerner G, Rosain J, Guérin A, et al. Inherited human IFN-γ deficiency underlies mycobacterial disease. *Journal of Clinical Investigation*. 2020;130(6):3158-3171. doi:10.1172/JCI135460
129. Bower H, Johnson S, Bangura MS, et al. Exposure-Specific and Age-Specific Attack Rates for Ebola Virus Disease in Ebola-Affected Households, Sierra Leone. *Emerg Infect Dis*. 2016;22(8):1403-1411. doi:10.3201/eid2208.160163
130. Khan A, Naveed M, Dur-e-Ahmad M, Imran M. Estimating the basic reproductive ratio for the Ebola outbreak in Liberia and Sierra Leone. *Infect Dis Poverty*. 2015;4(1):13. doi:10.1186/s40249-015-0043-3
131. Ebola Virus Disease among Male and Female Persons in West Africa. *New England Journal of Medicine*. 2016;374(1):96-98. doi:10.1056/NEJMc1510305
132. Hassouneh F, Goldeck D, Pera A, et al. Functional Changes of T-Cell Subsets with Age and CMV Infection. *Int J Mol Sci*. 2021;22(18):9973. doi:10.3390/ijms22189973
133. Kumar B V., Connors TJ, Farber DL. Human T Cell Development, Localization, and Function throughout Life. *Immunity*. 2018;48(2):202-213. doi:10.1016/j.immuni.2018.01.007
134. Ebola Virus Disease among Children in West Africa. *New England Journal of Medicine*. 2015;372(13):1274-1277. doi:10.1056/NEJMc1415318
135. McElroy AK, Erickson BR, Flietstra TD, et al. Biomarker Correlates of Survival in Pediatric Patients with Ebola Virus Disease. *Emerg Infect Dis*. 2014;20(10). doi:10.3201/eid2010.140430
136. Sun H, Wu Y, Zhang Y, Ni B. IL-10-Producing ILCs: Molecular Mechanisms and Disease Relevance. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.650200
137. Bruchez A, Sha K, Johnson J, et al. MHC class II transactivator CIITA induces cell resistance to Ebola virus and SARS-like coronaviruses. *Science (1979)*. 2020;370(6513):241-247. doi:10.1126/science.abb3753
138. Albakri K, Al-Hajali M, Saleh O, et al. Marburg virus disease treatments and vaccines: recent gaps and implications. *Annals of Medicine & Surgery*. 2023;85(2):328-330. doi:10.1097/MS9.0000000000000163
139. Lee A. Ansuvimab: First Approval. *Drugs*. 2021;81(5):595-598. doi:10.1007/s40265-021-01483-4
140. Markham A. REGN-EB3: First Approval. *Drugs*. 2021;81(1):175-178. doi:10.1007/s40265-020-01452-3