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REVIEW

The role of mononuclear phagocytes in Ebola virus infection

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Abstract

The filovirus, *Zaire Ebolavirus* (EBOV), infects tissue macrophages (Mϕs) and dendritic cells (DCs) early during infection. Viral infection of both cells types is highly productive, leading to increased viral load. However, virus infection of these two cell types results in different consequences for cellular function. Infection of Mϕs stimulates the production of proinflammatory and immunomodulatory cytokines and chemokines, leading to the production of a cytokine storm, while simultaneously increasing tissue factor production and thus facilitating disseminated intravascular coagulation. In contrast, EBOV infection of DCs blocks DC maturation and antigen presentation rendering these cells unable to communicate with adaptive immune response elements. Details of the known interactions of these cells with EBOV are reviewed here. We also identify a number of unanswered questions that remain about interactions of filoviruses with these cells.

KEYWORDS

ebola virus, filovirus, immune evasion, immune response, innate cell mediated immunity, viral pathogenesis

1 | INTRODUCTION

Zaire Ebola virus (EBOV) is a member of the *Ebolavirus* genus within the *Filoviridae* family of negative-strand RNA viruses which causes hemorrhagic fever in humans.[1] The genus was first identified in 1976 to cause two temporally overlapping outbreaks of hemorrhagic fever in Sudan and the DRC.[2] Since its discovery, five species of ebolaviruses have been identified, three of which, EBOV, Sudan (SUDV), and Bundibugyo (BDBV), have caused significant outbreaks in sub-Saharan Africa with case fatality rates as high as 90%.[3] EBOV emerged in West Africa resulting in the largest outbreak to date with over 28,000 reported cases and 11,000 fatalities[4] and, most recently, an outbreak in the Democratic Republic of the Congo has been reported (http://www.who.int/csr/don/30-may-2018-ebola-drc/en/). Despite the zoonotic nature of transmission of EBOV, the animal reservoir remains unknown, although fruit bats have been implicated as a natural reservoir and increased contact between humans and wild animals is thought to be the mechanism of zoonosis.[5]

Ebola virus disease (EVD) has been historically difficult to study due to the limited number of cases, lack of a known reservoir, and high degree of risk necessitating biosafety level 4 (BSL-4) containment. The current model of EVD is that the virus is transmitted through breaks in the skin and/or through mucosal membranes when the host comes in contact with bodily fluids (human to human) or tissues (animal to human) from an infected individual.[6] Once transmitted, EBOV is able to infect and replicate in the majority of human cells due to its use of a variety of host cell surface receptors as viral attachment and internalization factors and the ubiquitous expression of its endosomal receptor, Niemann Pick C1 (NPC1) (reviewed in Davey et al.[7]). Consistent with this, EBOV can be found by immunohistochemistry (IHC) in most major organs in fatal cases.[8] Once the host becomes viremic, virus replication proceeds largely unchecked by the host immune response due to the immune suppressive properties of the viral proteins VP35 and VP24[9–11] (reviewed in Prescott et al.[9]). Uncontrolled replication in major organ systems results in cell death, tissue damage, dysregulated cytokine/chemokine production, hemorrhage, and ultimately in organ failure.[12]

Abbreviations: BDBG, Bundibugyo Ebolavirus; BSL-4, Biosafety level 4; CLEC, C-type lectin; DC, Dendritic cell; DIC, Disseminated intravascular coagulation; EBOV, *Zaire Ebolavirus*; EVD, Ebolavirus disease; GP, Glycoprotein; HOPS, Homotypic fusion and vacuole protein-sorting complex; IHC, Immunohistochemistry; ISG, Interferon stimulated gene; Mϕ, Macrophage; MDM, Monocyte-derived macrophage; NHP, Non-human primate; NPC1, Niemann Pick C1; PBMC, Peripheral blood mononuclear cell; PS, Phosphatidylserine; RBD, Receptor binding domain; SUDV, Sudan Ebolavirus; TPC2, Two-pore calcium channel; VLP, Virus-like particle

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2 | MONONUCLEAR PHAGOCYTES: ORIGINS AND FUNCTIONS

Mononuclear phagocytes are composed of dendritic cell (DC), macrophage (Mϕ), and monocytic subsets.[13] DCs are primarily responsible for antigen presentation to lymphocytes, connecting innate and adaptive immunity. These cells sample their environment by endocytosing antigens, proteolytically processing those antigens and presenting the peptides on their surface in the context of MHC molecules. Some tissue DC populations are migratory with DCs trafficking from tissues to local lymph nodes for antigen presentation. A number of phenotypically distinct DCs exist that have distinct abilities to present antigens. Although tissue Mϕs can also present antigens, these cells primarily serve as scavengers and, depending on their cytokine environment, can produce robust levels of cytokines that are proinflammatory or immunomodulatory.[14] Tissue Mϕs are currently thought to arise from several different cellular origins with most that are present in tissues derived from self-renewing resident populations.[15] A small fraction of tissue Mϕ are thought to be derived from bone marrow derived monocytes which can seed tissues and differentiate into Mϕs.[15] Monocytes circulate in the blood and can home to tissues during infection. These can also be highly proinflammatory. Until recently, circulating monocytes were thought to mature into either DCs or Mϕs depending on their cytokine environment; however, transcriptome studies have firmly placed these cells within a monocyte/macrophage lineage. As discussed below, this recent assignment calls for reinterpretation of some earlier EBOV studies.

At early times during filovirus infection, tissue associated mononuclear phagocytes are thought to be the principal host cell targets as demonstrated in experimental infections of cynomolgous macaques, mice, and guinea pigs as well as in infected human tissue samples.[8,16–21] Consistent with these observations, the ability of EBOV to infect and replicate in Mϕs and DCs has also been confirmed in numerous tissue culture models.[22–25] Surprisingly, the role of circulating monocytes during filovirus infection is poorly studied and only recently examined.[26–28] However, these studies suggest that this mononuclear phagocytic population may also play an important role during infection.

Although it is clear that tissue Mϕs and DCs are permissive to EBOV in vivo and in vitro (reviewed in Martinez et al.[29]), many questions remain about interactions of filoviruses with these cells. Further, with the appreciation that bone marrow derived monocytes may also support EBOV infection,[26,27,30] traffic into tissues in response to pathogens, adapt to their new environment, and then disappear following resolution of infection,[31] the role of monocytes in filovirus infection and pathogenesis needs to be included in this discussion. It should be noted that in reviews, Mϕs and DCs are frequently grouped together as early and critically important cells when discussed in the context of filovirus infection, yet the impact of virus infection on the functionality of these cell populations differs. In turn, the impact of the maturation and activation of these cells on EBOV infection differs. When circulating monocytes are added to this group of cells, there is much that is not known about EBOV interaction with these cells. The interplay of each of these cell types with EBOV is the focus of this review.

3 | RECEPTORS ON DCS, MONOCYTES, AND Mϕ AND FILOVIRUS ENTRY AND REPLICATION

EBOV enters cells through a complex series of steps that can be broken down into relatively non-specific extracellular, and highly specific intracellular events. EBOV initially binds to the surface of host cells by engaging either C-type lectins (CLECs), which bind to N- or O-linked glycans on the heavily glycosylated Ebola glycoprotein, or phosphatidylserine (PS) receptors which mediate clearance of apoptotic bodies.[32–42,44,45] In the context of enveloped virus infections, PS receptors recognize PS in the host-derived outer membrane of the virion. A large number of PS receptors facilitate EBOV entry into cell lines. These include members of the TAM family of receptors, Axl, Mer, Tyro3, the T cell immunoglobulin mucin (TIM) family of receptors, TIM-1 and TIM-4, and additional receptors such as the scavenger receptor A. The CLEC receptors important for EBOV uptake include dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN), liver/lymph node-specific ICAM-3 grabbing non-integrin (L-SIGN), liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin), and macrophage galactose-type lectin (MGL).[32–35,43] Expression of these receptors on mononuclear phagocytes varies. For instance, whereas Mer is found on most, if not all, Mϕs,[46] TIM-4 is highly expressed on peritoneal Mϕs, but not on bone marrow derived Mϕs or monocyte-derived Mϕs.[47,48] Similarly, CLEC receptor expression on monocytes and Mϕ can be tissue specific[49] and a recent study by Harman et al. demonstrated that CLEC expression on DCs varies significantly between DC subsets, suggesting that receptor profiles are highly variable among susceptible cell populations.[50] Which cell surface receptors mediate filovirus entry into Mϕs and DCs in various organs remain to be fully defined and is discussed in more detail below. Further, signaling events associated with the use of these receptors likely are critical for regulating filovirus infection in different cell types, but have been poorly explored to date.[51,52] Whereas receptor redundancy suggests that individual receptors may be dispensable, a recent study using human monocyte derived macrophages (MDMs) found that siRNA-mediated knockdown of three receptors: Mer, integrin αV, and scavenger receptor A significantly reduced EBOV entry into MDMs.[45] They further found that Axl and TIM-1 expression is low on human MDMs and siRNA mediated knockdown of these receptors did not reduce EBOV entry, highlighting that these two well-established surface receptors for other cell types may not be important for EBOV infection of myeloid cells.[45]

Following virus/cell surface interactions, EBOV is internalized by macropinocytosis into endosomes, where the virion-associated glycoprotein (GP) is processed by endosomal proteases, such as cathepsin B and L, exposing the EBOV GP receptor-binding domain (RBD).[53–55] The newly exposed RBD binds to the late endosomal/lysosomal receptor NPC1, leading to subsequent fusion events and release of the viral genome into the cytoplasm.[56,57] Additional endosomal factors such as two-pore calcium channel (TPC2) and the homotypic fusion and vacuole protein-sorting (HOPS) complex also have been implicated in endosomal fusion events.[56,58]

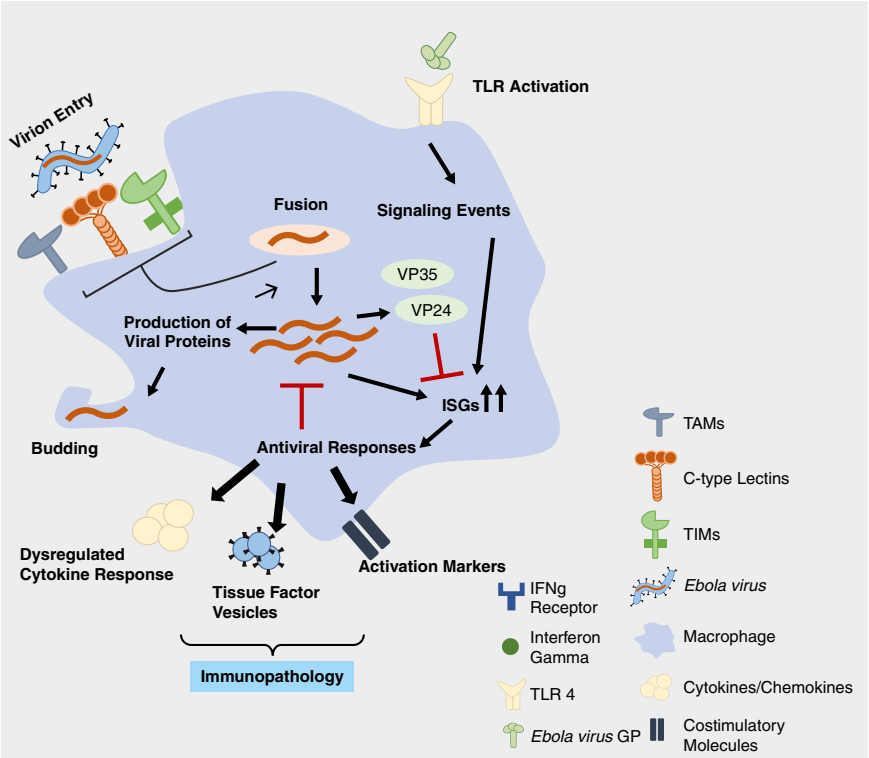


FIGURE 1 Immunopathology caused by EBOV infection of macrophages. Ebola virus enters macrophages utilizing surface receptors such as Mer, integrin αV, and scavenger receptor A. Likely additional receptors that are used are CLECs and other PS receptors. Also on the cell surface, EBOV GP interacts with TLR4, stimulating production of proinflammatory cytokines such as type I and II interferons that lead to ISG production. Entry via TAMs leads to upregulation of SOCS proteins which facilitate budding and inhibit TLR4-dependent antiviral responses. Upon release of viral genome into the cytoplasm, virus transcription and replication triggers interferon responses, production of cytokines/chemokines, tissue factor and upregulation of costimulatory molecules. Counteracting this, viral encoded proteins block interferon responses. The combination of these various events are thought to elicit the immunopathology associated with EVD

4 | EBOV ELICITS Mϕ IMMUNOPATHOLOGY

Filovirus replication in macrophages—and perhaps circulating monocytes recruited to sites of infection—results in dysregulated cytokine production, generating a "cytokine storm" consisting of both pro- and anti-inflammatory cytokines and leading to hypotensive crisis, and production of the vasoactive peptides, tissue factor and tumor necrosis factor (TNF), which facilitates coagulopathies such as disseminated intravascular coagulation (DIC) and hemorrhage.[59–62] Thus, EBOV activation of Mϕs and the subsequent production of proinflammatory cytokines in these cells is thought to contribute to the pathology of EVD rather than protecting the host (Fig. 1).[61–63] These observations were first made in the early 2000s when a flurry of studies found that monocytes and MDMs infected with either WT EBOV or EBOV pseudovirions rapidly produced a number of proinflammatory cytokines.[63–65] Later work used microarray analysis to identify cytokine and chemokine transcripts up-regulated in human MDMs infected with WT EBOV and virus-like particles (VLPs) and found 88 genes, including a number of cytokines and chemokines, were up-regulated as early as 6 h post infection.[24] This work corroborates prior studies in EVD patients which identified significant increases in serum protein levels of proinflammatory cytokines at the time of symptom onset.[66,67] Two recent studies that use next-generation sequencing (NGS) to analyze patient samples obtained during the 2013–2016 outbreak show that the response to EBOV in peripheral blood mononuclear cells (PBMCs) is in fact much more complex than initially hypothesized with a host of genes differentially expressed in fatal cases vs. survivors.[68,69] In addition to up-regulation and hypersecretion of pro- and anti-inflammatory chemokines and cytokines that were previous appreciated to occur,[67] changes in expression of a large number of previously unappreciated genes were identified. Expression of some of these correlated with survival.[68,69] Of note, many cytokines/chemokines, identified in previous studies to have altered expression during EBOV infection, were not identified in these studies; however, differences in approach as well as differences between transcriptional and translational changes may partially account for this.

A more recent study looked more specifically at transcript levels in PBMCs in an experimental macaque infection with EBOV and found over 100 genes up-regulated by EBOV infection, most of which were interferon stimulated genes (ISGs) and many of which were not reported previously to be up-regulated during EBOV infection.[70] Extensive production of ISGs during EBOV infection is somewhat surprising because two EBOV proteins, VP35 and VP24, serve as robust IFN antagonists.[10,11,71–75] However, as the authors suggest,[70] uninfected neighboring cells may be responsible for ISG production. Alternatively, infection of target cells with replication incompetent viral particles may elicit ISG production.

In combination, these studies suggest that EBOV infection causes significant changes in gene expression early in infection which likely results in the immunopathology associated with EVD. As tissue Mϕs and DCs are early EBOV targets and as EBOV has been shown to infect but not stimulate maturation or activation of DCs,[25] elevated cytokines at early time points are thus attributed primarily to Mϕs. Which specific subsets of tissue resident Mϕ and/or monocyte populations are responsible for the cytokine storm is not currently known. Targeted cell depletion studies would provide insights into the role of these different cell populations in tissue virus load, viremia, proinflammatory cytokine production and overall pathogenesis.

As EBOV actively inhibits type I and II interferon responses through several different VP35 and VP24 dependent mechanisms,[9–11,74,76,77] it is important to identify additional signaling pathways in macrophages and monocytes activated by EBOV that elicit cytokines and chemokines despite viral interference. The first insight into the mechanism underlying macrophage activation during EBOV infection came in a report which showed EBOV GP expressed on VLPs interact with TLR4 on a human monocyte cell line to induce pro-inflammatory cytokine production (i.e., IL-6, TNF, IFN-β) in a manner similar to LPS.[78] This interaction was found to be EBOV GP specific as VLPs lacking GP failed to induce a response. A subsequent study found that virus itself was not necessary for activation of TLR-4 on macrophages. Shed EBOV GP ectodomain, generated from virion- and cell-associated GP that is cleaved with cell surface proteases,[79] was able to activate uninfected macrophages in a TLR-4 dependent manner resulting in cytokine production (i.e., TNF, IL-1β, IL-6, IL-8, IL-12p40, IL-10) and up-regulation of co-stimulatory markers.[80] Consistent with this, a number of studies demonstrated that purified EBOV GP, EBOV GP constructs or EBOV VLPs administered to monocytes, macrophages or DCs leads to significant production of proinflammatory cytokines, chemokines and type I interferon responses.[78,80–83] Olejnik et al. examined signaling pathways involved in GP/TLR4-stimulated cytokine/chemokine production, implicating both NF-κB and IRF3 pathways.[81] Interestingly, the dimeric secreted form of GP (sGP), which is produced from the same gene as EBOV GP and, like shed GP, is present abundantly in infected blood, does not activate Mϕs.[80] The authors postulate this is due to differential glycosylation patterns between dimeric sGP and trimeric GP as TLR-4 interacts with glycosylated proteins and de-glycosylated shed GP lost the ability to activate TLR-4.[80] These findings are supported by an in vivo study showing that both BALB/c and C57BL/6 mice produced elevated cytokines (i.e., TNF, IL-1β, IL-6, IFN-γ, IL-2, IL-4, IL-12p70, IL-10) following administration of recombinant EBOV GP.[84] TLR-4 inhibitors blocked this cytokine production and inhibited macrophage trafficking to the draining lymph node and protected against lethal EBOV challenge.[85] As a number of different cell types in addition to macrophages and DCs express TLR4, many additional host cells may contribute to the TLR4-elicited cytokine storm that EBOV causes. Finally, in addition to TLR4, other currently unexplored EBOV/macrophage receptor interactions and subsequent signaling events may contribute to the production of the proinflammatory cytokines.[86,87]

A natural question that follows from these studies is how critical is myeloid cell activation to EBOV pathogenesis? Does the production of proinflammatory cytokines from these cells in response to EBOV contribute to virus-associated pathology? To address this, it was found that, unlike EBOV, infection with the non-pathogenic *Reston ebolavirus* (RESTV) did not activate TLR4 signaling in human MDMs or cytokine production.[81] This was further confirmed using inactivated RESTV as well as RESTV VLPs.[81] Although it is premature to suggest that lack of TLR4 signaling in macrophages fully explains the differences in pathogenicity between EBOV and RESTV, these studies collectively provide evidence that TLR4 activation on macrophages contributes to the immunopathology of EVD.

Whereas much attention has been given to the pro-inflammatory effects of EBOV, it is important to note that the reality is more of a tug-of-war, as EBOV employs a number of immune suppressive mechanisms as previously mentioned. In addition to interferon suppression mediated by EBOV VP24, VP35 and Marburg VP40,[9,88–90] suppression of TLR-elicited cytokine cascades occur following binding by virion-associated PS to the TAM receptors Tyro3, Axl, Mer.[51,91] The current consensus is that TAM signaling leads to up-regulation of SOCS1/SOCS3, which in turn dampens the innate immune response, preventing expression of host antiviral genes and proinflammatory cytokines/chemokines.[92] Also EBOV interactions with CLEC receptors may have immunosuppressive effects. Suppression of RIG-I mediated immunity occurs following binding of measles virus to the CLEC receptor DC-SIGN[93] and a similar immunosuppression of signaling following EBOV GP binding to DC-SIGN is possible. Thus, viruses such as the filoviruses that utilize these PS and CLEC receptors for cell entry would elicit reduced innate immune responses and thereby enhance infection. Consistent with this, it was shown that infection of bone marrow derived dendritic cells with several enveloped viruses, including EBOV, was significantly enhanced by TAM-mediated inhibition of TLR and IFN signaling.[51] It is reasonable to postulate that TAM signaling in macrophages may play an important, yet currently poorly appreciated, role in EBOV pathogenesis because macrophages up-regulate Mer expression during maturation,[22] EBOV uses Mer for entry into macrophages,[45] and SOCS1 and SOCS3 are increased upon infection of macrophages with EBOV.[78,94] In addition, the production of SOC3 through signaling pathways activated by TAM receptors has been shown to lead to enhanced EBOV budding,[94] providing an additional mechanism by which virus production may be increased.

5 | THE ROLE OF MONOCYTES DURING FILOVIRUS INFECTION

The contribution to virus load by infected peripheral blood monocytes within the PBMC population remains controversial within the field. Martinez et al. demonstrated that EBOV enters into human blood monocytes poorly and that maturation of monocytes into macrophages and/or dendritic cells is required for these cells to support significant levels of virus uptake.[22] The implication of this is that monocyte infection may not contribute substantially to systemic virus load. These authors showed that key EBOV entry factors, cathepsin B and NPC1, are up-regulated during the differentiation/maturation process and presumably an appropriate endosomal environment for filovirus entry is only achieved when sufficient amounts of these important endosomal trafficking proteins/complexes are available.[22] Further, expression of these host proteins are enhanced by EBOV infection.[22] These results are consistent with observations that mature tissue Mϕs and DCs are critical cell populations for support of EBOV replication.[16,17] However, it should be noted that a number of the early in vivo studies did not evaluate virus load present in PBMCs and it is possible that EBOV infection of circulating monocytes went unreported.

Other studies support a role for circulating PBMCs in active EBOV infection.[26–28,63,69] A recent longitudinal study in a patient with severe EVD demonstrated that virus load in PBMCs correlates well with serum RNA levels,[69] suggesting that virus replication in PBMCs contributes significantly to overall virus load. Consistent with the possibility that circulating monocytes support EBOV infection, Versteeg et al. showed that at later times during experimental infection (day 4–6) of EBOV (Makona) infection that peripheral blood monocyte numbers are decreased.[27] A similar loss of blood monocytes was observed in infected patient samples during the EBOV (Makona) epidemic in Guinea, with those patients with the highest viral loads having the lowest monocyte counts.[28] These data suggest that these cells are either recruited to other locations or depleted due to virus-induced cell killing. Additional studies that investigated the role of PBMCs in EBOV infection of NHPs demonstrated that monocytes purified from PBMCs contained the preponderance of EBOV transcripts that are found within blood leukocytes and this study suggests that infected blood monocytes may contribute significantly to overall virus load.[26] Consistent with the presence of significant EBOV load in blood monocytes, host genes associated with type I IFN signaling and inflammation are up-regulated in these cells. Thus, in contrast to the ex vivo Martinez et al. study, these results implicate blood monocytes as a potentially important host cell for virus replication and proinflammatory cytokines. It is important to acknowledge that differences in experimental approach (in vivo vs. ex vivo infection) and method of detection (viral transcript levels vs. viral entry) may partially account for these discrepancies. Additional studies directly comparing virus loads and cytokine profiles in circulating monocyte, monocytes in infected organs, and tissue Mϕs/DCs would provide insights into the importance of these different cell populations during EBOV infection.

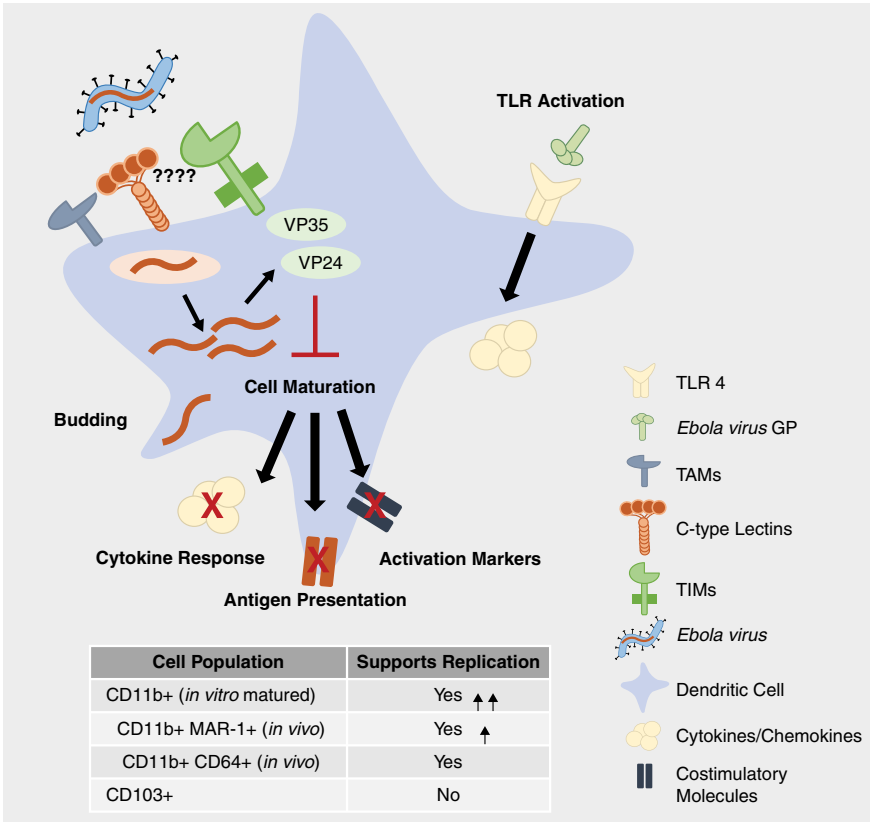
6 | INTERACTION OF EBOV WITH DCS

In studies looking at the effect of EBOV infection on DCs, almost all studies have used in vitro IL4/GM-CSF generated DCs that are derived from human blood monocytes or murine bone marrow derived monocytes.[22,25,72,75,80,87,95–98] However, a recently appreciated caveat to these studies is the observation that bone marrow monocytes matured in vitro produce a heterogeneous population of cells, with cultures containing both DCs and Mϕs.[99] Consistent with this, "in vitro generated DCs" derived from human monocytes are reported to be most similar to CD14+ tissue mononuclear phagocytes,[50] behave much like MDMs[100] and lack the potency of antigen presentation associated with in vivo derived DCs.[101] Thus, conclusions drawn from studies that use in vitro generated DCs may incompletely model findings obtained from in vivo DC population as these cells are likely not a pure DC population.

Nonetheless, these in vitro generated DCs have been shown to have decidedly different responses to EBOV infection than Mϕs. As noted above, studies with purified EBOV GP indicate that GP binding stimulates the maturation of DCs and the production of proinflammatory cytokines and chemokines.[80,87,96] However, EBOV infects DCs without stimulating maturation of these cells. This results in DCs with an immature phenotype that are not thought to produce cytokines/chemokines and poorly present antigen to lymphocytes. This aberrant behavior by DCs is thought to be due to virus expression of the host immune antagonists, VP35 and VP24[71–73,75,96] because mutagenesis of VP24 and VP35 within the EBOV genome results in dramatic up-regulation of DC activation markers, interferons, proinflammatory cytokines and chemokines.[73] Expression of these immunosuppressive proteins seems to disproportionately impact DCs as Mϕs are activated during EBOV infection (discussed above). Thus, from studies of in vitro generated DCs, it has been established that, whereas DCs serve as sites of virus replication and purified EBOV GP can elicit cytokine/chemokine production in DCs, these cells may not be significant contributors to the robust proinflammatory response observed during EBOV infection due to the effects of filovirus VP35 and VP24.[25,71–73,96] Instead, EBOV infection of DCs suppresses the important antigen presenting activities of these cells (Fig. 2).

Another caveat to these findings is that these observations are predicated on the assumption that virions entering DCs are infectious and thus producing VP35 and VP24 during virus replication. However, the production of abundant defective interfering (DI) particles would alter this scenario, with DI particles potentially eliciting proinflammatory responses, due to virus entry eliciting TLR4 signaling, but an absence of VP35 and VP24 production. Alfson et al. recently showed that viral stocks containing enhanced non-infectious particle-to-infectious particle ratios cause greater weight loss and worse clinical scores in NHPs.[102] Increased pathogenesis may be due to enhanced proinflammatory DC responses to the non-infectious particles. Studies utilizing primary DC populations obtained from human and murine tissues will be critical in validating and extending these observations.

Limited studies have looked at EBOV infection of tissue-associated DCs in vivo. These studies are challenging to perform with a BSL4 pathogen and, to date, have only been performed in mice, but similar studies are needed in other animal models such as infected NHPs. Studies using immature and/or mature DCs obtained from discarded human or animal model tissue skin may also provide important insights into EBOV/DC biology.[103] Ludtke et al. investigated DC populations that were infected following intranasal EBOV inoculation of mice,[98] detecting virus antigen positivity in CD11b+ DCs, but not CD103+ DCs, in lung at days 4–9 following infection. For closer inspection of the CD11b+ DC compartment, CD11b+ cells were stained for MAR-1 and CD64 to separate them into inflammatory monocyte-derived DCs and conventional lung-resident DCs, respectively.[104] Monocyte-derived DCs were more robustly infected at early, but not later, time points.[98] Because infected DCs are thought to traffic to regional lymph nodes, decreases in levels of EBOV infected inflammatory DCs at later time points may be due to trafficking of these cells, thereby facilitating dissemination of the virus throughout the body.[105,106] Alternatively, these cells may be depleted from the lung due to virus-induced cell killing. These were some of the first studies to address which mononuclear phagocyte populations support EBOV infection in vivo. The authors further investigated the contribution of inflammatory DCs in virus load. Somewhat surprisingly, loss of this permissive population within the lung at later times during infection had no effect on lung virus load, suggesting that other resident cells such



Cell Population	Supports Replication
CD11b+ (*in vitro* matured)	Yes ↑↑
CD11b+ MAR-1+ (*in vivo*)	Yes ↑
CD11b+ CD64+ (*in vivo*)	Yes
CD103+	No

FIGURE 2 Schematic of EBOV infection of dendritic cells. Ebola virus enters dendritic cells utilizing receptors that have yet to be defined but likely consist of CLECs and/or PS receptors. Upon release of virus into cells the antiviral proteins VP35 and VP24 prevent upregulation of costimulatory molecules, antigen presentation, and production of cytokines. In contrast recognition of EBOV GP by TLR4 directly activates dendritic cells. Importantly, not all dendritic cell populations are infected with EBOV (insert)

as alveolar macrophages are critically important for infection. Future investigations need to consider Mϕs, monocytes and DCs as unique, well-defined cell populations when looking at their roles in EBOV pathogenesis, and to account for all these populations when interpreting in vivo data.

Of note, the surface receptors on DCs that mediate EBOV adherence and internalization into the endosomal compartment have not been examined to date. Many DC populations express the CLEC receptors, MGL and LSECtin, and these specific receptors are known to serve as cell surface receptors for filoviruses.[34,35,43,107,108] However, the role of phagocytic receptors for filovirus uptake has yet to be explored on either in vitro or in vivo matured DCs.

7 | THE IMPACT OF MACROPHAGE POLARIZATION ON EBOV INFECTION

A growing body of research has shown that Mϕs exist as a spectrum of subtypes depending on their chemical micro-environment and that these populations differ greatly in their biologic properties (Fig. 3).[109] Whereas the nomenclature used to describe these cells is in flux, polarized or activated Mϕs are broadly divided into "classically activated" M1 or "alternatively-activated" M2 phenotypes based on their gene expression and role in maintaining homeostasis, with several subcategories described based on the exact stimulus used to induce polarization. Generally speaking, M1 polarized Mϕs are produced by proinflammatory stimuli such as IFN-γ or bacterial lipopolysaccharide. M1 Mϕs have elevated levels of expression of a number of proinflammatory cytokines, including IL-1β, TNF, IL-12, and IL-6, as well as ISGs in the case of IFN-γ stimulation. M1 Mϕs are thought to mediate clearance of pathogens. In contrast, M2 polarized Mϕs are elicited by a number of stimuli including IL-4, IL-13, tumor growth factor-β, dexamethasone and/or IL-10. M2 Mϕs produce a number of immunomodulatory compounds such as IL-10 and TGF-β and are thought to promote resolution of inflammation and wound healing.[110] Additionally, the concept of Mϕ activation status altering the host immune response has been explored in diseases such as diabetes, cancer, and atherosclerosis, where it has been shown that Mϕ populations are skewed to an M1 "pro-inflammatory" state in diabetes, an M2 "anti-inflammatory" state in the case of cancer, and both M1 and M2 populations in various aspects of atherosclerosis.[111–114] A number of studies have also investigated the role of Mϕ polarization in the context of infections with bacterial, parasitic and viral pathogens.[115–119] Viral pathogens such as HIV can modulate the status of macrophages to avoid the immune response or facilitate proliferation and dissemination, suggestive of the "evolutionary arms-race" between host and virus.[120] These effects are not unique to chronic viral infections, as it has been shown that influenza virus promotes M1 polarization of Mϕs and lung pathology is reduced in the presence of M2 cells.[118,121] Given the role of Mϕs as critical targets of EBOV and mediators of EVD, it is logical that the physiologic state of these cells may have significant impacts on infection and disease progression. Surprisingly, because macrophage production of dysregulated proinflammatory cytokines are thought to contribute to viral pathogenesis, evidence suggests that M1 polarization blocks EBOV infection and M2 polarization may enhance EBOV infection.

To date limited studies have explored the role of Mϕ polarization on EBOV infection. The first investigation showed that treatment of mice

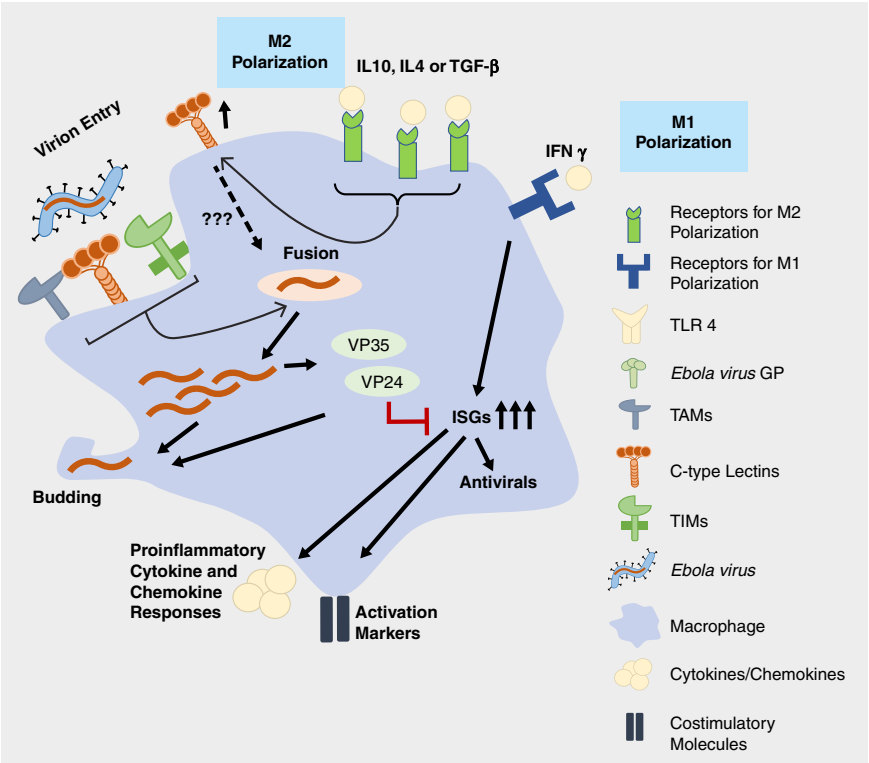


FIGURE 3 Macrophage polarization alters E8OV infection and replication in macrophages. Polarization/activation of tissue macrophages influences EBOV infection. Treatment of Mϕs with MI polarizing interferon gamma results in the transcription of ISGs. These host proteins include proinflammatory cytokines/chemokines, macrophage activation markers such as CD40 and intracellular proteins that have direct antiviral activities. At early times during EBOV infection, these events lead to reduced EBOV replication. Treatment of Mϕs with M2 polarizing agents, IL-4/-13, IL-10, orTGF-p, may enhance EBOV infection of macrophages, perhaps in part by increasing the expression of CLECs on the plasma membrane

with the M1 polarizing agent, IFN-γ, protected the mice from lethal challenge with mouse-adapted EBOV.[23] This protection was shown to be dose dependent, and effective when administered within a 24-h window prior to or after virus challenge, suggesting an effect on early cellular mediators of infection and disease such as Mϕs. Consistent with this possibility, in vitro studies with murine peritoneal Mϕs exposed to IFN-γ were protected from EBOV. Thus, IFN-γ-induction of M1 environment decreased permissiveness of an important cellular target of filoviruses, the macrophage. A number of ISGs up-regulated by the IFN-γ-treatment were identified to inhibit EBOV replication without impacting virus entry. These protective ISGs were not proinflammatory cytokines or chemokines, but intracellular proteins that likely function as antivirals; however, their mechanisms of protection have not been established. Thus, resistance to EBOV provided by IFN-γ may not be due to enhanced pro-inflammatory cytokines, but the production of innate antiviral responses within an early cellular target, Mϕs. Subsequent to this study, it was shown by another group that human MDMs treated with IFN-γ were also protected from WT EBOV infection in vitro, albeit to a lesser degree than reported in the earlier paper.[122]

Investigations into the effect of M2 polarization on EBOV infection are much more limited. A study by Martinez et al. touches on this question, suggesting that IL-4/IL-13 elicited polarization of human MDMs may modestly increase EBOV entry; however, statistical significance in the increase was not achieved in their studies.[22] Other studies have shown that stimulation of DCs and Mϕs with IL-4/IL-13 leads to up-regulation of DC-SIGN, a member of the CLEC receptors and known EBOV entry receptor which would be anticipated to enhance EBOV infection.[33,123]

From the limited studies to date, M1 Mϕs have been found to be resistant to EBOV infection, whereas the more immunomodulatory state of the M2 Mϕ may enhance EBOV infection. These results are far from conclusive, but emphasize the need for additional studies to help understand how cell polarization may impact EBOV infection and provide significant insights into potential therapeutics. Furthermore, one can easily envision a scenario where individuals are more or less susceptible to EVD depending on the transcriptional profiles of Mϕs and DCs at the site of infection.

8 | CONCLUDING REMARKS

Although our understanding of the mechanisms by which EBOV enters and replicates in mononuclear phagocytes is evolving rapidly, there are numerous gaps in our knowledge that need to be addressed. Of primary interest is investigation into the role of individual receptors on these cells to determine if there are receptors that would be amenable to therapeutic blockade. Independent of differences in receptor expression and innate immune responses elicited in the myeloid lineage, it is possible that additional events occurring later in the EBOV infectious cycle may be uniquely influenced by the monocyte/Mϕ/DC environment. For instance, cell specific rates of transcription and/or translation may impact viral replication. Additionally, virus packaging and budding may be influenced in a cell specific manner. These important questions have yet to be extensively explored in these cells. Additionally, whereas the vast majority of work has been done using either monocyte or bone marrow derived Mϕs and DCs, it is important to identify differences in susceptibility/permissivity of

various tissue resident populations which are likely to be among the first to be infected by and respond to EBOV. Further, the role of circulating monocytes in the blood and in tissues needs further exploration. The consequences of EBOV infection of these specific cell populations needs to be better understood. This ranges from the elucidation of impact of the maturation of these cells to the activation of innate immune responses and production of cytokines to the understanding of potential therapeutic strategies aimed at regulating these responses. Gaining such knowledge would facilitate preventative measures that are in development. Finally, it is important to consider the context in which EBOV infection occurs and how that may impact mononuclear phagocyte function and susceptibility.

AUTHORSHIP

K.R. and W.M. cowrote and edited this manuscript. K.R. generated the figures.

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DISCLOSURE

The authors declare no conflicts of interest.

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