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Immunological features underlying viral hemorrhagic fevers

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Several enveloped RNA viruses of the arenavirus, bunyavirus, filovirus and flavivirus families are associated with a syndrome known as viral hemorrhagic fever (VHF). VHF is characterized by fever, vascular leakage, coagulation defects and multi organ system failure. VHF is currently viewed as a disease precipitated by viral suppression of innate immunity, which promotes systemic virus replication and excessive proinflammatory cytokine responses that trigger the manifestations of severe disease. However, the mechanisms by which immune dysregulation contributes to disease remain poorly understood. Infection of nonhuman primates closely recapitulates human VHF, notably Ebola and yellow fever, thereby providing excellent models to better define the immunological basis for this syndrome. Here we review the current state of our knowledge and suggest future directions that will better define the immunological mechanisms underlying VHF.

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Introduction

Among the more well-known causes of VHF are the filoviruses Ebola virus (EBOV) and Marburg virus (MARV), the arenavirus Lassa virus, and the flaviviruses yellow fever (YFV) and dengue (DENV) virus. How diverse virus families with different replication strategies cause a similar clinical syndrome is incompletely understood, but several features typify these infections. The viruses effectively suppress innate antiviral defenses and replicate systemically to high titers; monocytes, macrophages and dendritic cells are targets of infection; a systemic cytokine storm occurs; and vascular leakage and hemorrhage may be seen. Nevertheless, specific details of the immunological underpinnings of VHF are lacking, and a unified view as to how the virus, the innate immune response and the adaptive immune response interact in the setting of VHF is also absent. Here, we argue that the availability of well-established nonhuman primate models of EBOV and YFV disease provide the necessary tools to define the immunological features common to VHFs, leading to a greater understanding of the syndrome and the suggestion of novel therapeutic approaches.

Detailed look at EBOV

The ebolaviruses and marburgviruses are enveloped viruses with non-segmented negative-sense single-stranded RNA genomes that belong to the family *Filoviridae*. There are 5 species of ebolavirus: Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Bundibugyo ebolavirus (BDBV), Tai Forrest ebolavirus (TAFV) and Reston ebolavirus (RESTV) and a single species of marburgvirus, Marburg marburgvirus (MARV). Only EBOV, SUDV, BDBV and MARV have been associated with outbreaks of severe disease and high mortality in humans. The most detailed descriptions of filovirus disease come from studies of EBOV and will form the main basis for our discussion of filovirus hemorrhagic fever.

Filovirus genomes possess 7 genes that encode: nucleoprotein (NP), viral protein of 35 kDa (VP35), VP40, glycoprotein (GP; mediates viral attachment and entry), VP30, VP24 and Large protein (L; the enzymatic component of the viral RNA-dependent RNA polymerase). The EBOV replication cycle takes place in the cytoplasm. Virus release occurs by budding from the plasma membrane in a process directed by the matrix protein VP40 and enhanced by other viral proteins, including GP.

Pathophysiology

Infections occur due to direct contact with infectious material, such as bodily fluids containing infectious virus. Airborne transmission is not thought to be a significant route of human infection, but aerosolized virus does cause rapidly lethal disease in experimentally infected nonhuman primates (reviewed in [1]). Following exposure, an incubation period of 2–21 days is followed by an abrupt but non-specific viral syndrome characterized by fever, chills and myalgia. As infection progresses, prostration, nausea, vomiting, abdominal pain and diarrhea appear. The final stages of disease are characterized by coagulopathy and vascular leakage resulting in hemorrhage and shock as reviewed in [2].

Many of the details of EBOV pathogenesis are derived from nonhuman primate studies, as they closely parallel severe human infections and are considered the 'gold-standard' model of EBOV disease (EVD). The hallmarks of EVD are high levels of systemic virus replication, cytokine production, liver damage, coagulopathy and lymphopenia [2]. Although filoviruses productively infect a variety of cell types, dendritic cells (DCs), macrophages and monocytes appear to be the preferential targets [2–5]. This may be due to first, viral GP interaction with lectins, such as dendritic-cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) on the surface of these cells [6–10], or second, phosphatidylserine on the surface of virus particles interacting, either directly or through an intermediate, with molecules such as TIM-1, TAM or αVβ3 and αVβ5 integrins [11–18]. Because these immune cells support productive viral infection and are capable of trafficking *in vivo*, their infection likely facilitates dissemination of the virus to lymph nodes and systemically [2,4,5,19•].

The dissemination of EBOV to hepatocytes, adrenal cortical cells and endothelial cells likely contributes to coagulopathy, which can result in hemorrhage and shock [4]. Virus-induced liver damage reduces production of coagulation factors, while infection of the adrenal gland reduces production of hormones that regulate blood pressure [4]. In addition, infected monocytes and macrophages produce proinflammatory mediators (IL-1β, IL-6, IL-8, IL-10, MIP-1β and TNFα), reactive oxygen species, nitric oxide, and tissue factor (TF) [20–27], which promote endothelial leakage and hypovolemia [28–31]. The cellular sensors and signaling pathways by which EBOV infection promotes production of cytokines and chemokines by monocytes are incompletely defined. *In vitro* studies demonstrate that extensive EBOV replication is not required to elicit cytokine production, but likely sustains the cytokine response (Figure 1).

Immune evasion

In contrast to monocytes/macrophages, EBOV infection of DCs is characterized by an inhibition of IFN-α/β and cytokine production, down-regulation of co-stimulatory molecules, and reduced ability to activate T cells [32,33•,34,35,36•]. The VP35 proteins target multiple innate immune signaling pathways to suppress IFN-α/β production and its antiviral effects [37–42,43••,44–47]. X-ray crystal structures demonstrate that the EBOV and MARV VP35s bind the phosphodiester backbone of dsRNA and that EBOV VP35 also 'caps' the ends of dsRNAs in a manner that could mask 5′-triphosphates [38,40,41,42,43••,48,49]. EBOV VP35 can also interact with cellular protein PACT to prevent PACT-mediated activation of RIG-I. Mutations in VP35 that disrupt interactions with dsRNA and PACT abrogate VP35 inhibition of IFN responses [36•,38,40,41,42,43••,48–53,54•]. Furthermore, mutations in VP35 impair virus replication in IFN-α/β competent cells and attenuate the virus *in vivo*, demonstrating a critical role for innate immune suppression for pathogenesis [52,53,54•].

In addition, EBOV and MARV block the Jak-STAT signaling pathways triggered when IFNs are added to cells, thereby disrupting the antiviral effects of these cytokines. EBOV VP24 blocks the nuclear accumulation of tyrosine phosphorylated STAT1 by binding to the NPI-1 subfamily of karyopherin alpha (KPNA) proteins [55–57,58••], whereas MARV VP40 blocks signaling by tyrosine kinase Jak1, preventing all the tyrosine phosphorylation events that typically occur after IFN addition to cells [59,60].

The impact of DC suppression on adaptive immunity *in vivo* remains to be determined, as virus-specific T cell responses develop in both EBOV-infected mice and people who survive infection [61,62••]. Moreover, lymphopenia is another common feature of EBOV infection, with loss of CD4 T cells, CD8 T cells and NK cells in mouse and nonhuman primate models [63,64] as well as human patients [65]. Cell loss occurs primarily via apoptosis and although the basis for this phenomenon is not yet clear, it is believed to be mediated by pro-inflammatory cytokines, NO and soluble FAS ligand produced by monocytes/macrophages [22,25,66–68].

Detailed look at YFV

Virus epidemiology, genetics and replication

YFV is endemic in central Africa and South America where it results in approximately 200,000 cases and 30,000 deaths annually [69]. YFV is an arbovirus that is spread via mosquitoes belonging to the genera *Haemagogus* and *Aedes*. YFV is maintained through two life cycles: in the urban cycle, YFV is transmitted between humans via *Aedes aegypti*; and in the jungle cycle, YFV transmission occurs between non-human primates via *Hemagogus* mosquitoes in South America and *Aedes africanus* in Africa while humans can be infected by mosquitoes that previously fed on an infected monkey [70,71].

Like other members of the *Flaviviridae* family, YFV is a single positive stranded RNA virus with an 11Kb genome composed of a 5′ non-coding region, a single open-reading frame (ORF), and a 3′ non-coding region. The ORF encodes 3 structural proteins (capsid (C), membrane (prM), and envelope (E)) and 7 nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) [72]. Virus proteins are processed after translation of the entire polyprotein within the rough endoplasmic reticulum (ER). The main structural protein is envelope, which is anchored in the lipid bilayer of the viral envelope and plays an important role in viral entry [73]. Nonstructural proteins are mainly involved in RNA replication and post-translational cleavage of the virus polyprotein [74].