Leading Edge
Review

Cell

Camouflage and Misdirection: The Full-On Assault of Ebola Virus Disease

John Misasi[1] and Nancy J. Sullivan[2,*]
[1]Boston Children's Hospital, Department of Medicine, Division of Infectious Diseases, Boston, MA 02115, USA
[2]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: njsull@mail.nih.gov
http://dx.doi.org/10.1016/j.cell.2014.10.006

Ebolaviruses cause a severe hemorrhagic fever syndrome that is rapidly fatal to humans and nonhuman primates. Ebola protein interactions with host cellular proteins disrupt type I and type II interferon responses, RNAi antiviral responses, antigen presentation, T-cell-dependent B cell responses, humoral antibodies, and cell-mediated immunity. This multifaceted approach to evasion and suppression of innate and adaptive immune responses in their target hosts leads to the severe immune dysregulation and "cytokine storm" that is characteristic of fatal ebolavirus infection. Here, we highlight some of the processes by which Ebola interacts with its mammalian hosts to evade antiviral defenses.

Introduction

The *Filoviridae* family consists of three genera: Marburgvirus, Ebolavirus, and the newly identified Cuevavirus. Within the Ebolavirus genus, there are five species, including Zaire ebolavirus, Sudan ebolavirus, Bundibugyo ebolavirus, Taï Forest ebolavirus, and Reston ebolavirus. Filoviruses were first identified as the causative agent of a hemorrhagic fever syndrome in Marburg, Germany in 1967. Nine years later, the first two ebolaviruses were described in the Democratic Republic of Congo (formerly Zaire) and Sudan. Since then, more than 30 Ebola virus disease outbreaks have infected thousands, with a mean case fatality rate of ~65% in humans (Hartman et al., 2010). The recent Ebola virus disease outbreak in West Africa began in March of 2014 and has thus far caused more than 8,000 confirmed and probable cases, with a case fatality rate of about 50% (for the latest information see: http://www.who.int/csr/disease/ebola/en/).

Virus Life Cycle

Ebola particles are enveloped, filamentous, and contain a monopartite negative-sense RNA genome. Though Ebola initially targets macrophages and dendritic cells, it is able to infect almost all cell types, with the exception of lymphocytes (Wool-Lewis and Bates, 1998; Yang et al., 1998). Virus particles have been proposed to attach to host cells through multiple plasma membrane surface-expressed proteins (e.g., C-type lectins, DC-SIGN, integrins, TIM-1, Axl) (Alvarez et al., 2002; Baribaud et al., 2002; Kondratowicz et al., 2011; Lin et al., 2003; Schornberg et al., 2009; Shimojima et al., 2006; Simmons et al., 2003; Takada et al., 2000). Once attached to the plasma membrane, the viral envelope glycoprotein induces particle uptake via macropinocytosis. The induction of macropinocytosis appears to be dependent on the action of cell surface proteins, including TIM-1 and Axl (Aleksandrowicz et al., 2011; Brindley et al., 2011; Hunt et al., 2011; Moller-Tank et al., 2013; Mulherkar et al., 2011; Nanbo et al., 2010; Quinn et al., 2009; Saeed et al., 2010; Shimojima et al., 2007, 2006; Wen et al., 2013). After uptake into macropinosomes, particles travel to low-pH compartments of late endosomes and lysosomes, where the viral envelope glycoprotein (GP) is proteolytically cleaved by endosomal cysteine proteases (i.e., cathepsin B and L). This cleavage removes a heavily glycosylated region from GP (Chandran et al., 2005; Dube et al., 2009; Hood et al., 2010; Misasi et al., 2012; Schornberg et al., 2006) and exposes a domain in GP that binds specifically to the endosomal/lysosomal resident filovirus receptor Niemann-Pick C1 protein (NPC1) (Carette et al., 2011; Côté et al., 2011). Though current evidence suggests that NPC1 binding may be sufficient to trigger fusion of the viral and cellular membranes (Miller et al., 2012), it is as yet unclear whether additional host proteins or intracellular conditions are necessary (e.g., reducing conditions, altered pH, additional protease cleavage) (Brecher et al., 2012; Chandran et al., 2005) (Figure 1, left).

Once the viral and internal cell membranes fuse, the virus particle uncoats and its anti-genome is transcribed into mRNA using nucleocapsid-associated viral proteins. The virus genome consists of seven viral genes—*VP24*, the nucleoprotein (*NP*), *VP30*, *VP35*, the matrix protein (*VP40*), the RNA-dependent RNA polymerase (*L*), and the *glycoprotein*—which are transcribed into mRNA, resulting in the production of at least ten proteins. Transcription of the genome is mediated via a complex of VP30, VP35, and the viral polymerase L bound to an NP-coated genome (Bharat et al., 2012; Hartlieb et al., 2003, 2007; Modrof et al., 2003; Mühlberger et al., 1999; Sanchez and Kiley, 1987; Sanchez et al., 1993). VP30 phosphorylation leads to its dissociation from the VP35/L complex and is the signal to switch from transcription to replication (Biedenkopf et al., 2013; Martinez et al., 2011a). Following this switch, virus genomes are replicated and coated by NP, VP24, VP30, and VP35 (Mühlberger et al., 1999). During assembly, L associates with the ribonucleoprotein complex through an interaction with VP35. The ribonucleoproteins then associate with the matrix

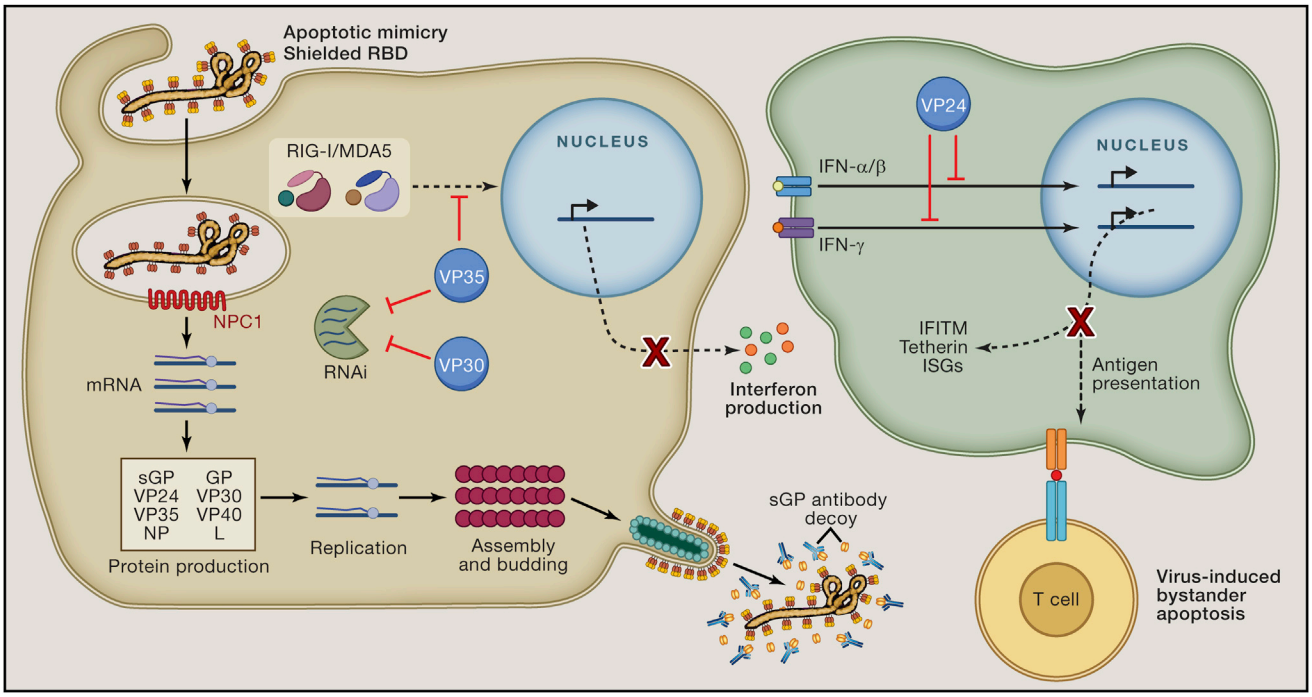

Figure 1. Ebolavirus Life Cycle and Immune Avoidance Mechanisms

(Left) Virus particles attach to cell surface, inducing macropinocytosis and virus uptake, possibly using apoptotic mimicry to suppress inflammatory responses. The particle is brought to a low pH compartment, where it is cleaved by cysteine proteases to reveal its NPC1 receptor-binding domain (RBD). Following fusion and uncoating, the viral genome is transcribed into mRNA and viral proteins produced. Eventually, a signal to begin genome replication occurs, followed by particle assembly and budding.

Expression and secretion of sGP serves as an antibody decoy for antibodies generated against GP. Viral proteins VP35, VP30, and VP24 are expressed and mediate innate immune avoidance in all cell types. (Left) VP35 interferes with RIG-I/MDA-5 signaling and induction of interferon. Additionally, VP35 and VP30 block the RNAi response against viral gene expression. (Right) VP24 acts to inhibit type I and II interferon (IFN) signaling. This prevents interferon-induced gene expression and, in antigen-presenting cells, blocks enhancement of antigen presentation to T cells.

protein VP40, and viral particles are extruded through the plasma membrane within lipid raft microdomain regions (Stahelin, 2014) (Figure 1, left).

Sneaking in with the Trash: Apoptotic Mimicry

Ebolavirus particles can be up to a micron in length, making it difficult for the viruses to enter via classic clathrin- or caveolin-mediated endocytosis pathways, perhaps explaining the requirement for macropinocytotic uptake. Interestingly, an increased amount of phosphatidylserine (PS) may be present on the surface of Ebola-virus-like particles (Jemielity et al., 2013; Moller-Tank et al., 2013). PS is a lipid that is primarily present on the inner leaflet of plasma membranes (Zachowski, 1993). Upon cell death via apoptosis, PS is exposed to the outer leaflets of plasma membranes and apoptotic bodies. This alerts nearby cells, including phagocytic cells, to begin "eating" the debris via macropinocytosis in a process that is mediated by TIM-1 and Axl and does not induce an inflammatory response (Biermann et al., 2013; Morizono and Chen, 2014; Zagórska et al., 2014). This suggests the possibility that, similar to other large viruses such as vaccinia virus (Mercer and Helenius, 2008), Ebola may induce macropinocytic uptake by appearing to be an apoptotic body to phagocytic cells. This "apoptotic mimicry" is anti-inflammatory and induces rapid uptake of a large virus into cells, thus avoiding humoral and cell surface immunity factors.

Snipping the Alarm Wire: Preventing the Interferon Alarm

Evasion of host innate immune responses is of particular importance to viruses, and many have evolved mechanisms to circumvent innate immunity. Ebola inhibits both type I and type II interferon responses in target cells, especially macrophages, monocytes, and dendritic cells. The ultimate result is a defect in dendritic cell maturation and diminished T-cell activation and proliferation along with apoptosis leading to lymphopenia, a key characteristic of Ebola virus disease. Studies in animal models and in tissue culture suggest that both pathogenesis and interferon antagonism are linked to VP35 and VP24 (Cilloniz et al., 2011; Ebihara et al., 2006; Hartman et al., 2008a, 2008b; Mateo et al., 2011; Prins et al., 2010; Reid et al., 2007).

VP35

VP35 is a viral polymerase cofactor that functions in RNA synthesis and has been proposed to link L to NP. In addition to these roles, VP35 plays a prominent role in Ebola's inhibition of α and β interferon induction through multiple mechanisms.

RIG-I and MDA-5 are innate pattern recognition receptors that detect foreign cytosolic RNA. RIG-I recognizes 5′-triphosphates

of blunt-ended RNA, and MDA-5 senses long double-stranded RNA (dsRNA). Both signal via the downstream adaptor IPS-1 (a.k.a. MAVS, VISA, Cardif), resulting in NF-κB, IRF-3, and IRF-7 activation with subsequent expression of type I interferon and proinflammatory cytokines. Activation of IRF-3/7 is the result of a signal cascade through which they are phosphorylated by TANK-binding kinase 1 (TBK-1) and IκB kinase-ε (IKKε) (Chiang et al., 2014). Early experiments determined that VP35 disrupted the RIG-I pathway by preventing IRF-3 phosphorylation (Basler et al., 2000, 2003). Later, VP35 was shown to interact with the N-terminal kinase domain of IKKε in preventing IRF-3 phosphorylation and acting as a decoy substrate for IKKε /TBK-1 kinases. Furthermore, binding of VP35 to IKKε prevents interactions with other proteins, including IRF-7 and IPS-1 (Prins et al., 2009). The net result of these interactions is inhibition of the induction of genes with interferon response promoters.

In addition to these downstream events in the RIG-I pathway, VP35 interacts with dsRNA to prevent RIG-I and MDA-5 responses (Cárdenas et al., 2006). Structural and biochemical studies revealed that VP35 contains a C-terminal interferon inhibitory domain (IID) with two clusters of basic amino acids. One cluster centers on residue R312 and participates in binding to dsRNA. Further analysis revealed that VP35 binds to blunt-ended dsRNA in a manner very similar to that seen with RIG-I (Cárdenas et al., 2006; Leung et al., 2009, 2010a). Structural studies of VP35 dsRNA binding are consistent with the finding that VP35 prevents both RIG-I and MDA-5 responses. Observations from RNA-bound and -unbound structures revealed that VP35 is able to bind both the phosphate backbone of dsRNA and end-capped RNA in VP35 dimers. Mutations of the basic patch centering on R312 abrogate dsRNA binding, and structural analysis suggests that R312 mutations disrupt VP35 dimerization (Kimberlin et al., 2010). Experiments using recombinant viruses incorporating mutant $VP35_{R312A}$ showed attenuation of virulence and impairment of both virus growth and interferon antagonism, suggesting that IID binding to dsRNA and VP35 dimerization play key roles in the virus life cycle and pathogenesis (Hartman et al., 2008a, 2008b; Kimberlin et al., 2010; Prins et al., 2010).

Interestingly, comparisons of VP35 IID from the pathogenic Zaire ebolavirus and Reston ebolavirus—thus far only pathogenic in monkeys—revealed a slight decrease in interferon antagonism and dsRNA binding by Reston. However, these decreases did not appear to contribute significantly to the differences in virulence between the Zaire and Reston viruses (Leung et al., 2010b). Furthermore, comparison of the structures of the Zaire and Reston VP35 did not reveal substantial differences between the dsRNA recognition mechanisms (Kimberlin et al., 2010). Together, these data suggest additional viral factors likely play a role in the differential host responses between these two viruses.

Recently, VP35 was found to interact with the PKR activator (PACT) (Fabozzi et al., 2011). In addition to having activity in RNA silencing and PKR activation, PACT binds to and activates RIG-I. Subsequent work showed that VP35 binding to PACT prevents PACT binding to RIG-I and inhibits RIG-I activation (Luthra et al., 2013). VP35 binding to PACT is mediated by the same central basic patch in IID that abrogates dsRNA binding described above, suggesting a possible role for dsRNA in this interaction. Surprisingly, PACT interaction with VP35 inhibits the binding of VP35 with L, causing a decreased efficiency of viral RNA synthesis and genome replication, a phenotype of "mutual antagonism" (Luthra et al., 2013). Taken together with previous data, these experiments point to the critical importance of VP35 antagonism of the RIG-I pathway during ebolavirus infection.

Additional VP35 interactions with cellular proteins have been explored using a yeast two-hybrid system. These studies found that VP35 interacts with IRF-7, Ubc9, and PIAS1 (Chang et al., 2009). Ubc9 and PIAS1 are key components of the small ubiquitin-related modifier (SUMO) system of posttranslational modification, which regulates a variety of cellular pathways and proteins. During SUMOylation, SUMO proteins are activated by SUMO-specific proteases and are transferred to a SUMO-conjugating E2 enzyme (e.g., Ubc9). Next, an E3 ligase, such as PIAS1, is used to transfer the SUMO domain to a lysine on the target protein (Wimmer et al., 2012). Studies with Ebola VP35 found that it was able to block CpG-induced interferon induction involving the IRF-3/7 pathway. Subsequent investigation revealed that PIAS1 is able to SUMOylate IRF-7. VP35 expression enhanced SUMOylation of IRF-7, leading to suppression of its activity and a decrease in interferon promoter activity. Similar findings were noted with IRF-3 following expression of VP35 (Chang et al., 2009). Thus, VP35-induced SUMOylation of IRF-3 and IRF-7 leads to a further reduction in interferon α/β gene transcription.

Arenaviruses (e.g., Lassa, LCMV) have taken a similar multipronged approach to preventing type I interferon responses. Lassa hemorrhagic fever has many characteristics similar to Ebola virus disease, including the absence of interferon production and lymphoid depletion. On the molecular level, arenaviruses have been shown to suppress interferon production by targeting both upstream (i.e., RIG-I/MDA-5) and downstream signaling events (i.e., IKKε interactions, IRF-3 phosphorylation) (Koma et al., 2013). In this way, each virus disrupts multiple access points in the pathways that lead to increased interferon production. Given the similarity in the clinical syndromes and the common approach to interferon antagonism, further investigations may provide insights into the underlying pathogenic mechanisms of hemorrhagic fever syndromes.

VP24

When innate immunity is intact, the host response to virus infection causes secretion of interferon in order to generate antiviral responses in neighboring cells, signal hematopoietic cell responses, and increase antigen presentation. Secreted interferon binds to type I and II interferon receptors, inducing signaling via adaptor proteins, and results in the phosphorylation and subsequent dimerization of signal transducer and activator of transcription (STAT) proteins (e.g., STAT-1, STAT-2). Next, dimerized phosphorylated STATs are transported to the nucleus where they bind to interferon response elements and induce gene expression (Ivashkiv and Donlin, 2014; Platanias, 2005). Given the importance of these pathways for inducing antiviral gene expression in response to interferon, they are commonly targeted by viruses. For example, Dengue virus blocks STAT-1 phosphorylation and acts to degrade STAT-2 via proteasomal degradation pathways (Green et al., 2014). Early experiments

with Ebola found that the virus not only blocked the production of interferon, but also inhibited cellular responses that normally result from both interferon α/β (type I) and interferon γ (type II) signaling. This signaling block was associated with the expression of the Ebola VP24 protein and later shown to prevent the nuclear accumulation of dimerized phosphorylated STAT-1 (Reid et al., 2006), which participates in both type I (i.e., STAT-1/STAT-2 phosphorylated-dimer) and type II (STAT-1/STAT-1 phosphorylated-dimer) signal propagation cascades (Ivashkiv and Donlin, 2014; Platanias, 2005).

Phosphorylated STAT-1 dimer transport to the nucleus is mediated by interactions with members of the nucleoprotein interactor 1 family (i.e., karyopherin-α1, -α5, or -α6). Karyopherin-α binds to nuclear localization signals (NLS) on cargo destined for the nucleus. Downstream interactions with karyopherin-β and other proteins allow the cargo to cross the nuclear membrane. Unlike most nuclear proteins, dimerized phosphorylated STAT-1 interacts with karyopherin-α through a unique noncanonical NLS, allowing it preferential access to the nucleus. Biochemical and structural studies have shown that VP24 binds to karyopherin-α in the noncanonical NLS-binding domain. This blocks phosphorylated STAT-1 dimer transport to the nucleus. Furthermore, VP24 binding to karyopherin-α does not appear to block access to the canonical NLS-binding site (Mateo et al., 2010; Reid et al., 2007; Xu et al., 2014). Therefore, the virus interfere with both type I and type II interferon signaling without disrupting routine trafficking to the nucleus of the infected cell.

Unphosphorylated STAT-1 is able to enter the nucleus using a karyopherin-α-independent mechanism, where it is able to induce and prolong the expression of interferon-induced immune regulatory genes (Cheon and Stark, 2009; Meyer and Vinkemeier, 2004). Biochemical and structural studies with STAT-1 C-terminal truncation mutant demonstrated that VP24 binds efficiently to STAT-1 lacking the tyrosine target for phosphorylation (Zhang et al., 2012). This suggests an additional mechanism unrelated to phosphorylated STAT-1 dimer nuclear transport by which VP24 is able to inhibit the induction of the interferon response (i.e., by blocking the action of both forms of STAT-1).

In addition to the Jak-STAT pathway, type I interferon receptors signal through the p38 MAP kinase pathway, where a signal cascade of MAP kinases results in the phosphorylation of p38-α (Ivashkiv and Donlin, 2014; Platanias, 2005). One study found that VP24 was able to block the interferon-β-induced phosphorylation of p38-α in HEK293T cells. However, this finding was cell type specific, and the blocking mechanism has yet to be demonstrated or generalized to cells that are primary targets of infection (e.g., macrophages, dendritic cells) (Halfmann et al., 2011).

Like VP35, VP24 has been linked to Ebola pathogenesis. In particular, experiments using mouse- and guinea-pig-adapted variants of Ebola demonstrated that VP24, in conjunction with other proteins such as NP, is critical to virus lethality (Cilloniz et al., 2011; Ebihara et al., 2006; Mateo et al., 2011; Reid et al., 2007). Structural analysis and comparison of Sudan and Reston VP24 identified two conserved structural pockets that contain residues implicated in the pathogenesis of VP24. Solvent exchange studies showed that this region may be near a proposed STAT-1-binding region on VP24, suggesting that it may play a role in STAT-1 binding and pathogenesis (Zhang et al., 2012).

Taken together with previous studies on karyopherin-α, VP24 plays a role in pathogenesis through the disruption of both type I and type II interferon signaling. Thus, Ebola not only diminishes the interferon alarm, but also inhibits the alarm response after it has been heard. This coordinated approach between VP35 and VP24 leads to a highly effective antagonism of the innate immune responses mediated by interferons.

Silence Will Fall: Disrupting the RNAi Response

Infection of cells by RNA viruses activates an RNA-specific inhibition (RNAi) pathway in host cells that silences viral gene expression by cleaving viral RNA into small interfering RNAs (siRNA) that bind and disrupt complementary RNA transcripts. HIV-1 Tat is thought to suppress RNAi responses by blocking Dicer activity, a key component of the RNA silencing complex. Ebola VP35 suppresses cellular RNAi silencing and can complement HIV-1 Tat$^-$ mutants. This suppression was lost when mutations were made in the dsRNA-binding domains of VP35, suggesting that VP35 may bind to siRNA or the dsRNA precursors of siRNA (Haasnoot et al., 2007). Though subsequent studies found that the C-terminal domain of VP35 bound to siRNA and not to dsRNA, the RNAi silencing suppression activity of VP35 did not correlate with binding to siRNA. It was hypothesized that suppression via RNA-binding-independent mechanisms could instead occur through binding to the RISC complex or sequestration of RISC complex proteins prior to their incorporation into the complex (Zhu et al., 2012). This theory is supported by prior experiments which found that VP35 interacts with transactivation response RNA-binding protein (TRBP) and PACT (Fabozzi et al., 2011), both components of the RISC RNAi complex and thus proposed to mediate the VP35-dependent RNA-silencing suppressor activity. Interestingly, another Ebola protein, VP30, also binds to components of the RISC complex and acts as a suppressor of RNAi silencing (Fabozzi et al., 2011). Similar to Ebola's tactics for interferon antagonism, the virus uses two different viral proteins to disrupt the mammalian RNAi innate immune response.

Plundering the Factories: Coopting Host Cellular Functions

Protein Translation

Host cells thwart viral infection by decreasing cellular protein synthesis in an effort to prevent or slow viral replication. This is accomplished through the double-stranded RNA-dependent protein kinase, PKR. Upon binding of dsRNA and activation, PKR phosphorylates the α subunit of the eukaryotic translation initiation factor-2 (eIF-2α). Ebola VP35 expression has been shown to block PKR activity and enhance expression of proteins after cells were treated with type I interferons. VP35 blocks PKR by impairing phosphorylation of both PKR and the eIF-2α. Current data suggest that this effect is mediated by the VP35 IID domain; however, further work to elucidate the molecular mechanism is required (Feng et al., 2007; Schümann et al., 2009).

RNA Stability and Replication

The heterogeneous nuclear protein complex C1/C2 proteins (hnRNP C1/C2) are typically found in the nucleus where they bind poly-U regions (>4) in mRNA and assist splicing prior to mRNA export to the cytoplasm. They have also been shown to

participate in cap-independent, IRES-dependent translation in the cytoplasm during mitosis (Shabman et al., 2011). Several viruses, including Dengue and poliovirus, have been found to co-opt this function as a means to enhance viral protein synthesis and replication (Brunner et al., 2005, 2010; Noisakran et al., 2008; Pettit Kneller et al., 2009). Likewise, Ebola VP24 expression causes the relocalization of hnRNP C1/C2 from the nucleus to the cytoplasm. Interestingly, hnRNP C1/C2 also binds to karyopherin-α via the same noncanonical NLS sequence STAT-1 and VP24 use, suggesting a possible mechanism for redistribution. It was hypothesized that, because the Ebola genomic and mRNA sequences contain many poly-U tracts, hnRNP C1/C2 may interact with Ebola RNA to stabilize viral mRNA and enhance genome replication (Shabman et al., 2011). Thus, through PKR inhibition and hnRNP C1/C2 relocalization, ebolaviruses co-opt cellular machinery to optimize translation of gene products and potentially prolong the half-life of viral messenger and genomic RNA.

Escape from Alcatraz: The Tetherin Release Program

Tetherin is a type I interferon-inducible cellular factor that has been shown to prevent enveloped virus budding from plasma membranes. Tetherin contains two membrane-anchored domains and is thought to mediate inhibition of budding by anchoring between the cellular and viral membranes (Neil et al., 2008). Tetherin was first described to antagonize retrovirus and HIV-1 particle budding. These studies found that particle budding was rescued by the expression of HIV-1 Vpu (Neil et al., 2008). When Ebola virus-like particles (VLP) were made with VP40 in the presence of overexpressed tetherin, a similar decrease in VLP release was noted and simultaneous expression of HIV-1 Vpu rescued particle release. VLPs with or without GP expression showed that GP antagonized tetherin activity and coimmunoprecipitated tetherin (Kaletsky et al., 2009). Recent experiments suggest that residues within the transmembrane domain of GP contribute to GP-dependent tetherin antagonism (Gnirß et al., 2014; Kühl et al., 2011). Mechanisms proposed for GP-tetherin antagonism include interference with tetherin integrity, steric hindrance by GP that interferes with the formation of the tetherin connection between the viral and cellular membranes, and GP-dependent exclusion of tetherin from the region of the plasma membrane from which Ebola virus particles bud (Kaletsky et al., 2009). In HIV-1, Vpu mediates cell-surface downregulation, relocalization, and degradation of tetherin (Lopez et al., 2012). However, Ebola GP does not appear to downregulate or alter tetherin localization (Lopez et al., 2010, 2012), suggesting a novel anti-tetherin mechanism.

The Host Strikes Back: Blocking the Entrances and Exits

The trade-off between efficient viral replication and pathogenicity is a delicate balance that, when mismatched, can lead to host demise. Therefore, viruses have evolved to suppress immune responses in a selective and regulated manner that facilitates controlled virus replication and progeny transmission. In this section, we highlight two interferon-inducible genes that, to date, are not known to be directly countered during Ebola infection and lead to decreased virus infectivity.

Interferon-induced transmembrane proteins (IFITMs) are broadly active interferon-responsive restriction factors that were first described as inhibitors of influenza A and have been shown to be active against many enveloped viruses, including Ebola and its cousin Marburg virus (Huang et al., 2011). IFITMs are found in multiple membrane surfaces along cellular uptake pathways and have been proposed to act by blocking virus entry (Huang et al., 2011; Perreira et al., 2013). Several antiviral mechanisms have been proposed, including cholesterol accumulation, inhibition of back fusion of virus containing vesicles in multivesicular bodies, changes to membrane fluid dynamics, or alterations in membrane curvature (i.e., restricting the capacity for fusion between the virus and cellular membranes) (Amini-Bavil-Olyaee et al., 2013; Perreira et al., 2013). Recent experiments with IFITM-3 and influenza A virus suggest that IFITM-3 acts by blocking an as yet undefined step between virus-cell membrane hemi-fusion and fusion pore formation (Desai et al., 2014). Thus, IFITM-3 allows the virus and celluar membrane lipids to mix but does not allow the formation of a complete fusion pore. This prevents the ribonucleoprotein core of the virus from gaining access to the cytoplasm and blocks infection. Future experiments will need to be performed to determine whether this mechanism is applicable to Ebola and how some enveloped viruses (i.e., arenaviruses) are able to avoid restriction by IFITMs.

Another interferon-stimulated gene that blocks Ebola infection is ISG-15, a broadly active antiviral gene product that, when conjugated to a target protein, causes alterations to target protein activity, cellular location, and stability. It has been shown to affect many viruses, including influenza A/B, hepatitis B, HIV-1, herpes simplex type 1, LCMV, and Ebola (Harty et al., 2009). In the case of Ebola, the antiviral activity of ISG-15 was linked to a disruption in VP40-mediated budding using VLP assays. Ebola budding is, in part, mediated by Nedd4 ubiquitination of VP40. Two groups found that ISG-15 interacts with Nedd4 and prevents the ubiquitination of VP40, leading to inefficient particle budding (Liu and Harty, 2010). Thus, together with IFITMs and tetherin, these observations suggest that interferon-stimulated genes have potent antiviral activities targeted against ebolavirus during both entry and egress.

The resistance of freshly isolated monocytes to Ebola illustrates one example in which these factors may be playing a role during infection. Experiments with Ebola GP-pseudotyped VLPs found that particles were able to bind, but not enter, freshly isolated monocytes. However, once the monocytes underwent differentiation, the previously bound VLPs were able to enter the monocytes, a result recapitulated using replicating Ebola viruses (Martinez et al., 2013). Detailed analysis found that, as the monocytes matured, IFITMs were downregulated while both cysteine protease cathepsin B and NPC1 expression were upregulated. These findings demonstrate how IFITM restriction factors might play a role in the relative resistance to infection of interferon-activated cells and also confirm the importance of NPC1 for Ebola infection.

Balancing the Scales of the Immune Response

A paradox in Ebola virus disease is that both survival and mortality are linked to the generation of strong immune signaling responses in the host. Survivors and asymptomatic patients

have increased numbers of T cells and an early cytotoxic T-cell-mediated response (Baize et al., 1999; Sanchez et al., 2004; Wauquier et al., 2010). Rapid uptake of Ebola by macrophages and dendritic cells not only results in translocation to lymphoid and peripheral tissues, but also may explain the deficit in nonsurvivors of inflammatory cytokine secretion needed early after exposure for the control of infection. Experiments using in-vitro-cultured monocyte-derived macrophages found that Ebola infection inhibited the secretion of TNF-α, IL-6, and IL-10, consistent with impairments observed in association with fatal disease outcome (Mahanty et al., 2003). Furthermore, dendritic cells exposed to either live or inactivated virus failed to upregulate molecules needed for T-cell co-stimulation, resulting in an inhibition of CD4 T-cell proliferation. Studies using blood samples obtained from infected human subjects found that IL-6 and TNF-α responses were higher in survivors than nonsurvivors at early time points. At later time points, IL-6 remained higher in survivors, but the difference from nonsurvivors was less dramatic due to increases in the latter group. In contrast to IL-6, TNF-α in nonsurvivors was much higher than survivors late after disease onset. It is interesting to note that IL-10 may play a critical role in modulating these responses. Although IL-10 was mildly elevated in survivors, likely as a feedback mechanism to control the inflammatory response, the increase was short lived, as would be expected once cytokine levels returned to normal. However, IL-10 was 6- to 10-fold higher in fatal cases and remained elevated until death. In addition, monocyte/macrophage activation as measured by neopterin levels was 2- to 10-fold higher and consistently elevated in fatal cases (Baize et al., 2002; Leroy et al., 2000, 2001), suggesting unregulated immune activation. Though more recent studies showed different patterns for specific cytokines (Wauquier et al., 2010), there is a general trend toward survivors having a short-lived, balanced pro- and anti-inflammatory response and nonsurvivors having a delayed and prolonged inflammatory response that leads to "cytokine storm."

Together, these observations suggest that subjects able to overcome innate immune response blocking by VP24, VP30, and VP35 are more likely to establish an early, balanced, and beneficial secretion of proinflammatory/anti-inflammatory cytokines. In contrast, when early host antiviral innate responses are blocked, uncontrolled virus replication and lysis of hematopoietic cells leads to late and unbalanced cytokine release, overall dysregulation of immune responses, and the development of advanced Ebola virus disease.

Camouflage and Misdirection: Avoiding Adaptive Immune Responses

A fatal irreversible consequence of hematopoietic cell destruction by Ebola is reduced antigen presentation. This outcome is consistent with the observation of poor IgG responses in fatal infection, whereas high levels of IgG are associated with survival (Baize et al., 1999; Ksiazek et al., 1999). Compounding inhibition of IgG production, Ebola has evolved multiple properties that could circumvent antibody effectiveness. Large filamentous virions containing high-density, stable glycoprotein present a potential obstacle for efficient inhibition by antibodies, and virus filament folding may create "pockets" of glycoprotein that are inaccessible to antibody binding. Furthermore, heavy glycosylation in the mucin-like domain of GP may limit access to critical epitopes required for efficient neutralization (Martinez et al., 2011b). Antibody access is restricted further due to rapid virion uptake via macropinocytosis and intracellular receptor binding. Additionally, ebolavirus hides its critical receptor binding domain beneath a glycan cap, with exposure and receptor binding occurring only after cathepsin-mediated removal of the cap. This protection of a critical functional domain is analogous to HIV, in which CD4 engagement of gp120 is required to expose the coreceptor-binding site (Harrison, 2008). Furthermore, the use of an intracellular receptor is a novel immune evasion strategy that may be important for other viruses (e.g., Lassa fever virus [Jae et al., 2014]).

In addition to membrane-anchored GP, the glycoprotein gene of Ebola encodes sGP, a 364 aa protein that is identical to GP in its 205 N-terminal residues but is secreted by infected cells and is not present in virions. The default transcript of the *glycoprotein* gene is surprisingly not the virion-associated GP trimer but instead dimeric sGP; the balance of expression of these two proteins is governed by polymerase stuttering at an RNA-editing site. This has led to speculation that sGP functions to modulate or misdirect host immune responses (Kindzelskii et al., 2000; Yang et al., 1998). The best evidence for this hypothesis comes from a recent paper showing in mice that sGP promotes immune evasion by serving as an antibody decoy for GP or by presenting alternative nonneutralizing antibody epitopes for the humoral immune response (Mohan et al., 2012). Remarkably, evidence suggests that about 80% of glycoprotein gene expression is sGP. This may be due, in part, to selective pressures seeking to balance toxicity due to cytopathic effects of GP with the requirement for GP on virus particles and avoidance of host immune responses directed against GP (Yang et al., 2000, Volchkova et al., 2011). Taken together, these data suggest that Ebola expression of sGP and GP is a tightly regulated process in which immune shielding and virus particle production are balanced via RNA editing.

In addition to immune evasion achieved through virion and GP structural characteristics, GP exhibits direct immunosuppressive properties (Chepurnov et al., 1999). sGP interacts with neutrophils and disrupts the linkage between Fc γ RIIIB and CR3 (Kindzelskii et al., 2000). Furthermore, the Ebola transmembrane glycoprotein, GP2, bears structural similarity to retroviral glycoproteins possessing an immunosuppressive peptide motif (Volchkov et al., 1992) that was found to inhibit lymphocyte activation and proliferation (Yaddanapudi et al., 2006). Of particular interest was the observation that the analogous immunosuppressive peptide from Reston virus was inhibitory only in macaque, but not human, peripheral blood cells, implicating this motif in Ebola pathogenicity.

Studies measuring in vivo antigen-specific T-cell responses are limited by the difficulty of obtaining and preserving viable lymphocyte samples from Ebola-infected subjects and survivors. Nonetheless, data suggest that, over the course of Ebola virus disease, there is a dramatic decrease in the absolute numbers of T cells due to bystander apoptosis. This impairs both direct cell-mediated killing of virus-infected cells and the T-cell-dependent antiviral antibody responses (Sanchez et al., 2004). Indirect measures of T-cell function based on serum

cytokine levels and RNA expression in isolated lymphocytes suggest, as with immunoglobulin levels, an association between intact cell-mediated immunity and survival (Baize et al., 1999; Ksiazek et al., 1999; Sanchez et al., 2004; Wauquier et al., 2010). In macaques, $CD8^+$ T cells are essential for vaccine-induced immune protection (Stanley et al., 2014; Sullivan et al., 2011). Humoral responses clearly play a beneficial role in containing virus but are not required for protection against some Ebola species (Hensley et al., 2010; Stanley et al., 2014; Sullivan et al., 2011). Antibodies participate in effective virus clearance but likely require the presence of intact host cell-mediated responses (Wong et al., 2012). One speculative interpretation of both human and macaque data is that immunoglobulin and innate immune responses participate in containing early viral loads but that cell-mediated immunity is needed for efficient virus clearance. The interplay and necessity of broad immune response mechanisms is also suggested by Ebola evolution of strategies to counteract each of these host antiviral defenses. Taken together, these data suggest a complex interplay between pro- and anti-inflammatory factors, resulting in either a balanced immune response and host survival or dysregulation and death.

Summary

Fatal Ebola infection is marked by a catastrophic failure of innate and adaptive immunity that is mediated by virus-encoded proteins as well as properties associated with virus structure. At the heart of Ebola-induced immune dysregulation is a multipronged attack on host antiviral immunity. Early and coordinated disruption of host innate responses by VP24, VP30, and VP35 leads to elevated levels of virus replication, a cascade of inappropriately timed cytokine release, and death of both antigen-presenting and -responding immune cells (Figure 1). This results in a poorly activated adaptive immune response that is further compromised by the induction of lymphocyte apoptosis and antibody decoy mechanisms. In incidental hosts, this multifaceted approach to subversion of the immune system results in high mortality that would be expected to limit virus persistence in the absence of a distinct reservoir species.

Since first being identified almost 40 years ago, the genome of Ebola has shown remarkable stability, an unusual feature for RNA viruses with an error-prone polymerase. This suggests that Ebola is highly adapted in its reservoir host. One intriguing hypothesis is that additional host restriction factors limit pathogenicity in a natural reservoir species. These factors would play a role in the balance between immunity and sustained viral replication, allowing the virus to propagate and persist over time in the reservoir species. Outside this reservoir, sustained large outbreaks such as the one in West Africa may lead to ebolavirus adaptations being observed longitudinally, resulting in altered pathogenicity as the virus adapts to humans. Overall, the multifaceted approach of Ebola to selectively regulate immune responses and its variable pathogenicity in different host species makes this virus both scientifically interesting and a challenging foe.

ACKNOWLEDGMENTS

The authors thank B. Hartman for graphics and A. Tislerics for editing the manuscript. This work was supported by the Intramural Research Program of the US NIH NIAID Vaccine Research Center, NIH grant K08-AI079381, and a Boston Children's Hospital Faculty Development Fellowship.

REFERENCES

Aleksandrowicz, P., Marzi, A., Biedenkopf, N., Beimforde, N., Becker, S., Hoenen, T., Feldmann, H., and Schnittler, H.-J. (2011). Ebola virus enters host cells by macropinocytosis and clathrin-mediated endocytosis. J. Infect. Dis. *204* (*Suppl 3*), S957–S967.

Alvarez, C.P., Lasala, F., Carrillo, J., Muñiz, O., Corbí, A.L., and Delgado, R. (2002). C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. J. Virol. *76*, 6841–6844.

Amini-Bavil-Olyaee, S., Choi, Y.J.J., Lee, J.H.H., Shi, M., Huang, I.-C., Farzan, M., and Jung, J.U.U. (2013). The antiviral effector IFITM3 disrupts intracellular cholesterol homeostasis to block viral entry. Cell Host Microbe *13*, 452–464.

Baize, S., Leroy, E.M., Georges-Courbot, M.C., Capron, M., Lansoud-Soukate, J., Debré, P., Fisher-Hoch, S.P., McCormick, J.B., and Georges, A.J. (1999). Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. Nat. Med. *5*, 423–426.

Baize, S., Leroy, E.M., Georges, A.J., Georges-Courbot, M.C., Capron, M., Bedjabaga, I., Lansoud-Soukate, J., and Mavoungou, E. (2002). Inflammatory responses in Ebola virus-infected patients. Clin. Exp. Immunol. *128*, 163–168.

Baribaud, F., Pöhlmann, S., Leslie, G., Mortari, F., and Doms, R.W. (2002). Quantitative expression and virus transmission analysis of DC-SIGN on monocyte-derived dendritic cells. J. Virol. *76*, 9135–9142.

Basler, C.F., Wang, X., Mühlberger, E., Volchkov, V., Paragas, J., Klenk, H.D., García-Sastre, A., and Palese, P. (2000). The Ebola virus VP35 protein functions as a type I IFN antagonist. Proc. Natl. Acad. Sci. USA *97*, 12289–12294.

Basler, C.F., Mikulasova, A., Martinez-Sobrido, L., Paragas, J., Mühlberger, E., Bray, M., Klenk, H.-D., Palese, P., and García-Sastre, A. (2003). The Ebola virus VP35 protein inhibits activation of interferon regulatory factor 3. J. Virol. *77*, 7945–7956.

Bharat, T.A., Noda, T., Riches, J.D., Kraehling, V., Kolesnikova, L., Becker, S., Kawaoka, Y., and Briggs, J.A. (2012). Structural dissection of Ebola virus and its assembly determinants using cryo-electron tomography. Proc. Natl. Acad. Sci. USA *109*, 4275–4280.

Biedenkopf, N., Hartlieb, B., Hoenen, T., and Becker, S. (2013). Phosphorylation of Ebola virus VP30 influences the composition of the viral nucleocapsid complex: impact on viral transcription and replication. J. Biol. Chem. *288*, 11165–11174.

Biermann, M., Maueröder, C., Brauner, J.M., Chaurio, R., Janko, C., Herrmann, M., and Muñoz, L.E. (2013). Surface code—biophysical signals for apoptotic cell clearance. Phys. Biol. *10*, 065007.

Brecher, M., Schornberg, K.L., Delos, S.E., Fusco, M.L., Saphire, E.O., and White, J.M. (2012). Cathepsin cleavage potentiates the Ebola virus glycoprotein to undergo a subsequent fusion-relevant conformational change. J. Virol. *86*, 364–372.

Brindley, M.A., Hunt, C.L., Kondratowicz, A.S., Bowman, J., Sinn, P.L., McCray, P.B., Jr., Quinn, K., Weller, M.L., Chiorini, J.A., and Maury, W. (2011). Tyrosine kinase receptor Axl enhances entry of Zaire ebolavirus without direct interactions with the viral glycoprotein. Virology *415*, 83–94.

Brunner, J.E., Nguyen, J.H.C., Roehl, H.H., Ho, T.V., Swiderek, K.M., and Semler, B.L. (2005). Functional interaction of heterogeneous nuclear ribonucleoprotein C with poliovirus RNA synthesis initiation complexes. J. Virol. *79*, 3254–3266.

Brunner, J.E., Ertel, K.J., Rozovics, J.M., and Semler, B.L. (2010). Delayed kinetics of poliovirus RNA synthesis in a human cell line with reduced levels of hnRNP C proteins. Virology *400*, 240–247.

Cárdenas, W.B., Loo, Y.-M., Gale, M., Jr., Hartman, A.L., Kimberlin, C.R., Martínez-Sobrido, L., Saphire, E.O., and Basler, C.F. (2006). Ebola virus

VP35 protein binds double-stranded RNA and inhibits alpha/beta interferon production induced by RIG-I signaling. J. Virol. *80*, 5168–5178.

Carette, J.E., Raaben, M., Wong, A.C., Herbert, A.S., Obernosterer, G., Mulherkar, N., Kuehne, A.I., Kranzusch, P.J., Griffin, A.M., Ruthel, G., et al. (2011). Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature *477*, 340–343.

Chandran, K., Sullivan, N.J., Felbor, U., Whelan, S.P., and Cunningham, J.M. (2005). Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. Science *308*, 1643–1645.

Chang, T.-H., Kubota, T., Matsuoka, M., Jones, S., Bradfute, S.B., Bray, M., and Ozato, K. (2009). Ebola Zaire virus blocks type I interferon production by exploiting the host SUMO modification machinery. PLoS Pathog. *5*, e1000493.

Cheon, H., and Stark, G.R. (2009). Unphosphorylated STAT1 prolongs the expression of interferon-induced immune regulatory genes. Proc. Natl. Acad. Sci. USA *106*, 9373–9378.

Chepurnov, A.A., Tuzova, M.N., Ternovoy, V.A., and Chernukhin, I.V. (1999). Suppressive effect of Ebola virus on T cell proliferation in vitro is provided by a 125-kDa GP viral protein. Immunol. Lett. *68*, 257–261.

Chiang, J.J., Davis, M.E., and Gack, M.U. (2014). Regulation of RIG-I-like receptor signaling by host and viral proteins. Cytokine Growth Factor Rev. http://dx.doi.org/10.1016/j.cytogfr.2014.06.005.

Cilloniz, C., Ebihara, H., Ni, C., Neumann, G., Korth, M.J., Kelly, S.M., Kawaoka, Y., Feldmann, H., and Katze, M.G. (2011). Functional genomics reveals the induction of inflammatory response and metalloproteinase gene expression during lethal Ebola virus infection. J. Virol. *85*, 9060–9068.

Côté, M., Misasi, J., Ren, T., Bruchez, A., Lee, K., Filone, C.M., Hensley, L., Li, Q., Ory, D., Chandran, K., and Cunningham, J. (2011). Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. Nature *477*, 344–348.

Desai, T.M., Marin, M., Chin, C.R., Savidis, G., Brass, A.L., and Melikyan, G.B. (2014). IFITM3 restricts influenza A virus entry by blocking the formation of fusion pores following virus-endosome hemifusion. PLoS Pathog. *10*, e1004048.

Dube, D., Brecher, M.B., Delos, S.E., Rose, S.C., Park, E.W., Schornberg, K.L., Kuhn, J.H., and White, J.M. (2009). The primed ebolavirus glycoprotein (19-kilodalton GP1,2): sequence and residues critical for host cell binding. J. Virol. *83*, 2883–2891.

Ebihara, H., Takada, A., Kobasa, D., Jones, S., Neumann, G., Theriault, S., Bray, M., Feldmann, H., and Kawaoka, Y. (2006). Molecular determinants of Ebola virus virulence in mice. PLoS Pathog. *2*, e73.

Fabozzi, G., Nabel, C.S., Dolan, M.A., and Sullivan, N.J. (2011). Ebolavirus proteins suppress the effects of small interfering RNA by direct interaction with the mammalian RNA interference pathway. J. Virol. *85*, 2512–2523.

Feng, Z., Cerveny, M., Yan, Z., and He, B. (2007). The VP35 protein of Ebola virus inhibits the antiviral effect mediated by double-stranded RNA-dependent protein kinase PKR. J. Virol. *81*, 182–192.

Gnirß, K., Fiedler, M., Krämer-Kühl, A., Bolduan, S., Mittler, E., Becker, S., Schindler, M., and Pöhlmann, S. (2014). Analysis of determinants in filovirus glycoproteins required for tetherin antagonism. Viruses *6*, 1654–1671.

Green, A.M., Beatty, P.R., Hadjilaou, A., and Harris, E. (2014). Innate immunity to dengue virus infection and subversion of antiviral responses. J. Mol. Biol. *426*, 1148–1160. http://dx.doi.org/10.1016/j.jmb.2013.11.023.

Haasnoot, J., de Vries, W., Geutjes, E.-J., Prins, M., de Haan, P., and Berkhout, B. (2007). The Ebola virus VP35 protein is a suppressor of RNA silencing. PLoS Pathog. *3*, e86.

Halfmann, P., Neumann, G., and Kawaoka, Y. (2011). The Ebolavirus VP24 protein blocks phosphorylation of p38 mitogen-activated protein kinase. J. Infect. Dis. *204* (*Suppl 3*), S953–S956.

Harrison, S.C. (2008). Viral membrane fusion. Nat. Struct. Mol. Biol. *15*, 690–698.

Hartlieb, B., Modrof, J., Mühlberger, E., Klenk, H.-D., and Becker, S. (2003). Oligomerization of Ebola virus VP30 is essential for viral transcription and can be inhibited by a synthetic peptide. J. Biol. Chem. *278*, 41830–41836.

Hartlieb, B., Muziol, T., Weissenhorn, W., and Becker, S. (2007). Crystal structure of the C-terminal domain of Ebola virus VP30 reveals a role in transcription and nucleocapsid association. Proc. Natl. Acad. Sci. USA *104*, 624–629.

Hartman, A.L., Ling, L., Nichol, S.T., and Hibberd, M.L. (2008a). Whole-genome expression profiling reveals that inhibition of host innate immune response pathways by Ebola virus can be reversed by a single amino acid change in the VP35 protein. J. Virol. *82*, 5348–5358.

Hartman, A.L., Bird, B.H., Towner, J.S., Antoniadou, Z.-A., Zaki, S.R., and Nichol, S.T. (2008b). Inhibition of IRF-3 activation by VP35 is critical for the high level of virulence of ebola virus. J. Virol. *82*, 2699–2704.

Hartman, A.L., Towner, J.S., and Nichol, S.T. (2010). Ebola and marburg hemorrhagic fever. Clin. Lab. Med. *30*, 161–177.

Harty, R.N., Pitha, P.M., and Okumura, A. (2009). Antiviral activity of innate immune protein ISG15. J. Innate Immun. *1*, 397–404.

Hensley, L.E., Mulangu, S., Asiedu, C., Johnson, J., Honko, A.N., Stanley, D., Fabozzi, G., Nichol, S.T., Ksiazek, T.G., Rollin, P.E., et al. (2010). Demonstration of cross-protective vaccine immunity against an emerging pathogenic Ebolavirus Species. PLoS Pathog. *6*, e1000904.

Hood, C.L., Abraham, J., Boyington, J.C., Leung, K., Kwong, P.D., and Nabel, G.J. (2010). Biochemical and structural characterization of cathepsin L-processed Ebola virus glycoprotein: implications for viral entry and immunogenicity. J. Virol. *84*, 2972–2982.

Huang, I.-C., Bailey, C.C., Weyer, J.L., Radoshitzky, S.R., Becker, M.M., Chiang, J.J., Brass, A.L., Ahmed, A.A., Chi, X., Dong, L., et al. (2011). Distinct patterns of IFITM-mediated restriction of filoviruses, SARS coronavirus, and influenza A virus. PLoS Pathog. *7*, e1001258.

Hunt, C.L., Kolokoltsov, A.A., Davey, R.A., and Maury, W. (2011). The Tyro3 receptor kinase Axl enhances macropinocytosis of Zaire ebolavirus. J. Virol. *85*, 334–347.

Ivashkiv, L.B., and Donlin, L.T. (2014). Regulation of type I interferon responses. Nat. Rev. Immunol. *14*, 36–49.

Jae, L.T., Raaben, M., Herbert, A.S., Kuehne, A.I., Wirchnianski, A.S., Soh, T.K., Stubbs, S.H., Janssen, H., Damme, M., Saftig, P., et al. (2014). Virus entry. Lassa virus entry requires a trigger-induced receptor switch. Science *344*, 1506–1510.

Jemielity, S., Wang, J.J., Chan, Y.K., Ahmed, A.A., Li, W., Monahan, S., Bu, X., Farzan, M., Freeman, G.J., Umetsu, D.T., et al. (2013). TIM-family proteins promote infection of multiple enveloped viruses through virion-associated phosphatidylserine. PLoS Pathog. *9*, e1003232.

Kaletsky, R.L., Francica, J.R., Agrawal-Gamse, C., and Bates, P. (2009). Tetherin-mediated restriction of filovirus budding is antagonized by the Ebola glycoprotein. Proc. Natl. Acad. Sci. USA *106*, 2886–2891.

Kimberlin, C.R., Bornholdt, Z.A., Li, S., Woods, V.L., Jr., MacRae, I.J., and Saphire, E.O. (2010). Ebolavirus VP35 uses a bimodal strategy to bind dsRNA for innate immune suppression. Proc. Natl. Acad. Sci. USA *107*, 314–319.

Kindzelskii, A.L., Yang, Z., Nabel, G.J., Todd, R.F., 3rd, and Petty, H.R. (2000). Ebola virus secretory glycoprotein (sGP) diminishes Fc gamma RIIIB-to-CR3 proximity on neutrophils. J. Immunol. *164*, 953–958.

Koma, T., Huang, C., Kolokoltsova, O.A., Brasier, A.R., and Paessler, S. (2013). Innate immune response to arenaviral infection: a focus on the highly pathogenic New World hemorrhagic arenaviruses. J. Mol. Biol. *425*, 4893–4903.

Kondratowicz, A.S., Lennemann, N.J., Sinn, P.L., Davey, R.A., Hunt, C.L., Moller-Tank, S., Meyerholz, D.K., Rennert, P., Mullins, R.F., Brindley, M., et al. (2011). T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebolavirus and Lake Victoria Marburgvirus. Proc. Natl. Acad. Sci. USA *108*, 8426–8431.

Ksiazek, T.G., Rollin, P.E., Williams, A.J., Bressler, D.S., Martin, M.L., Swanepoel, R., Burt, F.J., Leman, P.A., Khan, A.S., Rowe, A.K., et al. (1999). Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995. J. Infect. Dis. *179* (*Suppl 1*), S177–S187.

Kühl, A., Banning, C., Marzi, A., Votteler, J., Steffen, I., Bertram, S., Glowacka, I., Konrad, A., Stürzl, M., Guo, J.-T., et al. (2011). The Ebola virus glycoprotein

and HIV-1 Vpu employ different strategies to counteract the antiviral factor tetherin. J. Infect. Dis. *204* (*Suppl 3*), S850–S860.

Leroy, E.M., Baize, S., Volchkov, V.E., Fisher-Hoch, S.P., Georges-Courbot, M.C., Lansoud-Soukate, J., Capron, M., Debré, P., McCormick, J.B., and Georges, A.J. (2000). Human asymptomatic Ebola infection and strong inflammatory response. Lancet *355*, 2210–2215.

Leroy, E.M., Baize, S., Debre, P., Lansoud-Soukate, J., and Mavoungou, E. (2001). Early immune responses accompanying human asymptomatic Ebola infections. Clin. Exp. Immunol. *124*, 453–460.

Leung, D.W., Ginder, N.D., Fulton, D.B., Nix, J., Basler, C.F., Honzatko, R.B., and Amarasinghe, G.K. (2009). Structure of the Ebola VP35 interferon inhibitory domain. Proc. Natl. Acad. Sci. USA *106*, 411–416.

Leung, D.W., Prins, K.C., Borek, D.M., Farahbakhsh, M., Tufariello, J.M., Ramanan, P., Nix, J.C., Helgeson, L.A., Otwinowski, Z., Honzatko, R.B., et al. (2010a). Structural basis for dsRNA recognition and interferon antagonism by Ebola VP35. Nat. Struct. Mol. Biol. *17*, 165–172.

Leung, D.W., Shabman, R.S., Farahbakhsh, M., Prins, K.C., Borek, D.M., Wang, T., Mühlberger, E., Basler, C.F., and Amarasinghe, G.K. (2010b). Structural and functional characterization of Reston Ebola virus VP35 interferon inhibitory domain. J. Mol. Biol. *399*, 347–357.

Lin, G., Simmons, G., Pöhlmann, S., Baribaud, F., Ni, H., Leslie, G.J., Haggarty, B.S., Bates, P., Weissman, D., Hoxie, J.A., and Doms, R.W. (2003). Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR. J. Virol. *77*, 1337–1346.

Liu, Y., and Harty, R.N. (2010). Viral and host proteins that modulate filovirus budding. Future Virol. *5*, 481–491.

Lopez, L.A., Yang, S.J., Hauser, H., Exline, C.M., Haworth, K.G., Oldenburg, J., and Cannon, P.M. (2010). Ebola virus glycoprotein counteracts BST-2/Tetherin restriction in a sequence-independent manner that does not require tetherin surface removal. J. Virol. *84*, 7243–7255.

Lopez, L.A., Yang, S.J., Exline, C.M., Rengarajan, S., Haworth, K.G., and Cannon, P.M. (2012). Anti-tetherin activities of HIV-1 Vpu and Ebola virus glycoprotein do not involve removal of tetherin from lipid rafts. J. Virol. *86*, 5467–5480.

Luthra, P., Ramanan, P., Mire, C.E., Weisend, C., Tsuda, Y., Yen, B., Liu, G., Leung, D.W., Geisbert, T.W., Ebihara, H., et al. (2013). Mutual antagonism between the Ebola virus VP35 protein and the RIG-I activator PACT determines infection outcome. Cell Host Microbe *14*, 74–84.

Mahanty, S., Hutchinson, K., Agarwal, S., McRae, M., Rollin, P.E., and Pulendran, B. (2003). Cutting edge: impairment of dendritic cells and adaptive immunity by Ebola and Lassa viruses. J. Immunol. *170*, 2797–2801.

Martinez, M.J., Volchkova, V.A., Raoul, H., Alazard-Dany, N., Reynard, O., and Volchkov, V.E. (2011a). Role of VP30 phosphorylation in the Ebola virus replication cycle. J. Infect. Dis. *204* (*Suppl 3*), S934–S940.

Martinez, O., Tantral, L., Mulherkar, N., Chandran, K., and Basler, C.F. (2011b). Impact of Ebola mucin-like domain on antiglycoprotein antibody responses induced by Ebola virus-like particles. J. Infect. Dis. *204* (*Suppl 3*), S825–S832.

Martinez, O., Johnson, J.C., Honko, A., Yen, B., Shabman, R.S., Hensley, L.E., Olinger, G.G., and Basler, C.F. (2013). Ebola virus exploits a monocyte differentiation program to promote its entry. J. Virol. *87*, 3801–3814.

Mateo, M., Reid, S.P., Leung, L.W., Basler, C.F., and Volchkov, V.E. (2010). Ebolavirus VP24 binding to karyopherins is required for inhibition of interferon signaling. J. Virol. *84*, 1169–1175.

Mateo, M., Carbonnelle, C., Reynard, O., Kolesnikova, L., Nemirov, K., Page, A., Volchkova, V.A., and Volchkov, V.E. (2011). VP24 is a molecular determinant of Ebola virus virulence in guinea pigs. J. Infect. Dis. *204* (*Suppl 3*), S1011–S1020.

Mercer, J., and Helenius, A. (2008). Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells. Science *320*, 531–535.

Meyer, T., and Vinkemeier, U. (2004). Nucleocytoplasmic shuttling of STAT transcription factors. Eur. J. Biochem. *271*, 4606–4612.

Miller, E.H., Obernosterer, G., Raaben, M., Herbert, A.S., Deffieu, M.S., Krishnan, A., Ndungo, E., Sandesara, R.G., Carette, J.E., Kuehne, A.I., et al. (2012). Ebola virus entry requires the host-programmed recognition of an intracellular receptor. EMBO J. *31*, 1947–1960.

Misasi, J., Chandran, K., Yang, J.-Y., Considine, B., Filone, C.M., Côté, M., Sullivan, N., Fabozzi, G., Hensley, L., and Cunningham, J. (2012). Filoviruses require endosomal cysteine proteases for entry but exhibit distinct protease preferences. J. Virol. *86*, 3284–3292.

Modrof, J., Becker, S., and Mühlberger, E. (2003). Ebola virus transcription activator VP30 is a zinc-binding protein. J. Virol. *77*, 3334–3338.

Mohan, G.S., Li, W., Ye, L., Compans, R.W., and Yang, C. (2012). Antigenic subversion: a novel mechanism of host immune evasion by Ebola virus. PLoS Pathog. *8*, e1003065.

Moller-Tank, S., Kondratowicz, A.S., Davey, R.A., Rennert, P.D., and Maury, W. (2013). Role of the phosphatidylserine receptor TIM-1 in enveloped-virus entry. J. Virol. *87*, 8327–8341.

Morizono, K., and Chen, I.S.Y. (2014). Role of phosphatidylserine receptors in enveloped virus infection. J. Virol. *88*, 4275–4290.

Mühlberger, E., Weik, M., Volchkov, V.E., Klenk, H.D., and Becker, S. (1999). Comparison of the transcription and replication strategies of marburg virus and Ebola virus by using artificial replication systems. J. Virol. *73*, 2333–2342.

Mulherkar, N., Raaben, M., de la Torre, J.C., Whelan, S.P., and Chandran, K. (2011). The Ebola virus glycoprotein mediates entry via a non-classical dynamin-dependent macropinocytic pathway. Virology *419*, 72–83.

Nanbo, A., Imai, M., Watanabe, S., Noda, T., Takahashi, K., Neumann, G., Halfmann, P., and Kawaoka, Y. (2010). Ebolavirus is internalized into host cells via macropinocytosis in a viral glycoprotein-dependent manner. PLoS Pathog. *6*, e1001121.

Neil, S.J.D., Zang, T., and Bieniasz, P.D. (2008). Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature *451*, 425–430.

Noisakran, S., Sengsai, S., Thongboonkerd, V., Kanlaya, R., Sinchaikul, S., Chen, S.-T., Puttikhunt, C., Kasinrerk, W., Limjindaporn, T., Wongwiwat, W., et al. (2008). Identification of human hnRNP C1/C2 as a dengue virus NS1-interacting protein. Biochem. Biophys. Res. Commun. *372*, 67–72.

Perreira, J.M., Chin, C.R., Feeley, E.M., and Brass, A.L. (2013). IFITMs restrict the replication of multiple pathogenic viruses. J. Mol. Biol. *425*, 4937–4955.

Pettit Kneller, E.L., Connor, J.H., and Lyles, D.S. (2009). hnRNPs Relocalize to the cytoplasm following infection with vesicular stomatitis virus. J. Virol. *83*, 770–780.

Platanias, L.C. (2005). Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat. Rev. Immunol. *5*, 375–386.

Prins, K.C., Cárdenas, W.B., and Basler, C.F. (2009). Ebola virus protein VP35 impairs the function of interferon regulatory factor-activating kinases IKKepsilon and TBK-1. J. Virol. *83*, 3069–3077.

Prins, K.C., Delpeut, S., Leung, D.W., Reynard, O., Volchkova, V.A., Reid, S.P., Ramanan, P., Cárdenas, W.B., Amarasinghe, G.K., Volchkov, V.E., and Basler, C.F. (2010). Mutations abrogating VP35 interaction with double-stranded RNA render Ebola virus avirulent in guinea pigs. J. Virol. *84*, 3004–3015.

Quinn, K., Brindley, M.A., Weller, M.L., Kaludov, N., Kondratowicz, A., Hunt, C.L., Sinn, P.L., McCray, P.B., Jr., Stein, C.S., Davidson, B.L., et al. (2009). Rho GTPases modulate entry of Ebola virus and vesicular stomatitis virus pseudotyped vectors. J. Virol. *83*, 10176–10186.

Reid, S.P., Leung, L.W., Hartman, A.L., Martinez, O., Shaw, M.L., Carbonnelle, C., Volchkov, V.E., Nichol, S.T., and Basler, C.F. (2006). Ebola virus VP24 binds karyopherin alpha1 and blocks STAT1 nuclear accumulation. J. Virol. *80*, 5156–5167.

Reid, S.P., Valmas, C., Martinez, O., Sanchez, F.M., and Basler, C.F. (2007). Ebola virus VP24 proteins inhibit the interaction of NPI-1 subfamily karyopherin alpha proteins with activated STAT1. J. Virol. *81*, 13469–13477.

Saeed, M.F., Kolokoltsov, A.A., Albrecht, T., and Davey, R.A. (2010). Cellular entry of ebola virus involves uptake by a macropinocytosis-like mechanism and subsequent trafficking through early and late endosomes. PLoS Pathog. *6*, e1001110.

Sanchez, A., Kiley, M.P., Holloway, B.P., and Auperin, D.D. (1993). Sequence analysis of the Ebola virus genome: organization, genetic elements, and comparison with the genome of Marburg virus. Virus Res. *29*, 215–240.

Sanchez, A.A.J., Lukwiya, M., Bausch, D., Mahanty, S., Sanchez, A.J., Wagoner, K.D., and Rollin, P.E. (2004). Analysis of human peripheral blood samples from fatal and nonfatal cases of Ebola (Sudan) hemorrhagic fever: cellular responses, virus load, and nitric oxide levels. J. Virol. *78*, 10370–10377.

Sanchez, A., and Kiley, M.P. (1987). Identification and analysis of Ebola virus messenger RNA. Virology *157*, 414–420.

Schornberg, K., Matsuyama, S., Kabsch, K., Delos, S., Bouton, A., and White, J. (2006). Role of endosomal cathepsins in entry mediated by the Ebola virus glycoprotein. J. Virol. *80*, 4174–4178.

Schornberg, K.L., Shoemaker, C.J., Dube, D., Abshire, M.Y., Delos, S.E., Bouton, A.H., and White, J.M. (2009). Alpha5beta1-integrin controls ebolavirus entry by regulating endosomal cathepsins. Proc. Natl. Acad. Sci. USA *106*, 8003–8008.

Schümann, M., Gantke, T., and Mühlberger, E. (2009). Ebola virus VP35 antagonizes PKR activity through its C-terminal interferon inhibitory domain. J. Virol. *83*, 8993–8997.

Shabman, R.S., Gulcicek, E.E., Stone, K.L., and Basler, C.F. (2011). The Ebola virus VP24 protein prevents hnRNP C1/C2 binding to karyopherin α1 and partially alters its nuclear import. J. Infect. Dis. *204* (*Suppl 3*), S904–S910.

Shimojima, M., Takada, A., Ebihara, H., Neumann, G., Fujioka, K., Irimura, T., Jones, S., Feldmann, H., and Kawaoka, Y. (2006). Tyro3 family-mediated cell entry of Ebola and Marburg viruses. J. Virol. *80*, 10109–10116.

Shimojima, M., Ikeda, Y., and Kawaoka, Y. (2007). The mechanism of Axl-mediated Ebola virus infection. J. Infect. Dis. *196* (*Suppl 2*), S259–S263.

Simmons, G., Reeves, J.D., Grogan, C.C., Vandenberghe, L.H., Baribaud, F., Whitbeck, J.C., Burke, E., Buchmeier, M.J., Soilleux, E.J., Riley, J.L., et al. (2003). DC-SIGN and DC-SIGNR bind ebola glycoproteins and enhance infection of macrophages and endothelial cells. Virology *305*, 115–123.

Stahelin, R.V. (2014). Membrane binding and bending in Ebola VP40 assembly and egress. Front. Microbiol. *5*, 300.

Stanley, D.A., Honko, A.N., Asiedu, C., Trefry, J.C., Lau-Kilby, A.W., Johnson, J.C., Hensley, L., Ammendola, V., Abbate, A., Grazioli, F., et al. (2014). Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. Nat. Med. Published online September 7, 2014. http://dx.doi.org/10.1038/nm.3702.

Sullivan, N.J., Hensley, L., Asiedu, C., Geisbert, T.W., Stanley, D., Johnson, J., Honko, A., Olinger, G., Bailey, M., Geisbert, J.B., et al. (2011). CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. Nat. Med. *17*, 1128–1131.

Takada, A., Watanabe, S., Ito, H., Okazaki, K., Kida, H., and Kawaoka, Y. (2000). Downregulation of beta1 integrins by Ebola virus glycoprotein: implication for virus entry. Virology *278*, 20–26.

Volchkov, V.E., Blinov, V.M., and Netesov, S.V. (1992). The envelope glycoprotein of Ebola virus contains an immunosuppressive-like domain similar to oncogenic retroviruses. FEBS Lett. *305*, 181–184.

Volchkova, V.A., Dolnik, O., Martinez, M.J., Reynard, O., and Volchkov, V.E. (2011). Genomic RNA editing and its impact on Ebola virus adaptation during serial passages in cell culture and infection of guinea pigs. J. Infect. Dis. *204* (*Suppl 3*), S941–S946.

Wauquier, N., Becquart, P., Padilla, C., Baize, S., and Leroy, E.M. (2010). Human fatal zaire ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis. PLoS Negl. Trop. Dis. *4*, 4.

Wen, Z., Zhao, B., Song, K., Hu, X., Chen, W., Kong, D., Ge, J., and Bu, Z. (2013). Recombinant lentogenic Newcastle disease virus expressing Ebola virus GP infects cells independently of exogenous trypsin and uses macropinocytosis as the major pathway for cell entry. Virol. J. *10*, 331.

Wimmer, P., Schreiner, S., and Dobner, T. (2012). Human pathogens and the host cell SUMOylation system. J. Virol. *86*, 642–654.

Wong, G., Richardson, J.S., Pillet, S., Patel, A., Qiu, X., Alimonti, J., Hogan, J., Zhang, Y., Takada, A., Feldmann, H., et al. (2012). Immune parameters correlate with protection against ebola virus infection in rodents and nonhuman primates. Sci. Transl. Med. *4*, 158ra146.

Wool-Lewis, R.J., and Bates, P. (1998). Characterization of Ebola virus entry by using pseudotyped viruses: identification of receptor-deficient cell lines. J. Virol. *72*, 3155–3160.

Xu, W., Edwards, M.R.R., Borek, D.M.M., Feagins, A.R.R., Mittal, A., Alinger, J.B.B., Berry, K.N.N., Yen, B., Hamilton, J., Brett, T.J.J., et al. (2014). Ebola virus VP24 targets a unique NLS binding site on karyopherin alpha 5 to selectively compete with nuclear import of phosphorylated STAT1. Cell Host Microbe *16*, 187–200.

Yaddanapudi, K., Palacios, G., Towner, J.S., Chen, I., Sariol, C.A., Nichol, S.T., and Lipkin, W.I. (2006). Implication of a retrovirus-like glycoprotein peptide in the immunopathogenesis of Ebola and Marburg viruses. FASEB J. *20*, 2519–2530.

Yang, Z., Delgado, R., Xu, L., Todd, R.F., Nabel, E.G., Sanchez, A., and Nabel, G.J. (1998). Distinct cellular interactions of secreted and transmembrane Ebola virus glycoproteins. Science *279*, 1034–1037.

Yang, Z.Y., Duckers, H.J., Sullivan, N.J., Sanchez, A., Nabel, E.G., and Nabel, G.J. (2000). Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. Nat. Med. *6*, 886–889.

Zachowski, A. (1993). Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. Biochem. J. *294*, 1–14.

Zagórska, A., Través, P.G., Lew, E.D., Dransfield, I., and Lemke, G. (2014). Diversification of TAM receptor tyrosine kinase function. Nat. Immunol. *15*, 920–928.

Zhang, A.P.P., Bornholdt, Z.A., Liu, T., Abelson, D.M., Lee, D.E., Li, S., Woods, V.L., Jr., and Saphire, E.O. (2012). The ebola virus interferon antagonist VP24 directly binds STAT1 and has a novel, pyramidal fold. PLoS Pathog. *8*, e1002550.

Zhu, Y., Cherukuri, N.C., Jackel, J.N., Wu, Z., Crary, M., Buckley, K.J., Bisaro, D.M., and Parris, D.S. (2012). Characterization of the RNA silencing suppression activity of the Ebola virus VP35 protein in plants and mammalian cells. J. Virol. *86*, 3038–3049.