The contribution of the endothelium to the development of coagulation disorders that characterize Ebola hemorrhagic fever in primates

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Summary
Recently, there have been substantial developments in the understanding of Ebola hemorrhagic fever pathogenesis, but there are still major gaps. These infections occur in underdeveloped areas of the world, and much of our knowledge of naturally occurring disease is derived from sporadic outbreaks that occurred decades in the past. Recently conducted laboratory animal studies have provided insight into Ebola pathogenesis and may help guide clinical investigations of disease using contemporary methodologies that were not available previously. A better understanding of the relevant host and viral factors that influence clinical and virologic outcome will be critical to our ability to combat this aggressive pathogen. This article reviews the most relevant information relating to the postulated pathogenesis of this disease, focusing on the role of the endothelium in contributing to the coagulation disorders that characterize Ebola hemorrhagic fever in primates. Some of the remaining and key unanswered questions relating to the role of the vascular system in the pathogenesis of this disease, that need to be addressed in further research, are highlighted.

Keywords
Ebola virus, filovirus, hemorrhagic fever, pathogenesis

Ebola virus (EBOV) infection causes hemorrhagic fever (HF) with high mortality rates in humans and nonhuman primates. Currently, there are no vaccines or therapies approved for human use. Outbreaks of EBOV have been frequent, largely confined to remote locations in Africa, and quarantine of sick patients has been effective in controlling epidemics. Despite the relatively low global occurrence of cases, EBOV has gained notoriety largely because of the aggressive nature by which it causes disease in primates and because it is considered to have potential as a bioweapon (1).

EBOV belongs to the family Filoviridae, which is comprised of filamentous, enveloped, non-segmented negative-sense RNA viruses (reviewed in (2)). The family Filoviridae is divided into two genera: Marburgvirus (MARV) and Ebola virus (EBOV). The Marburgvirus genus contains a single species: Lake Victoria marburgvirus (LVMARV). The Ebola virus genus is subdivided into four distinct species: Ivory Coast ebolavirus (ICEBOV), Reston ebolavirus (REBOV), Sudan ebolavirus (SEBOV), and Zaire ebolavirus (ZEBOV). ZEBOV and SEBOV are important human pathogens with case fatality rates frequently ranging between 70 and nearly 90% in ZEBOV outbreaks and around 50% for SEBOV episodes. Limited data suggest that REBOV is non-pathogenic in humans while the pathogenic potential of ICEBOV is unknown as there has only been a single, nonfatal human case.

EBOV infections are usually the most severe of those caused by the viruses of lethal hemorrhagic disease in humans. Clinical symptoms appear suddenly after an incubation period of 2 to 21 days (3). Common presenting complaints include high fever, chills, malaise, and myalgia. As the disease progresses, there is evidence of multisystemic involvement, and manifestations include prostration, anorexia, vomiting, nausea, abdominal pain, diarrhea, shortness of breath, sore throat, hypotension, edema, confusion, and coma (2, 4–9). Abnormalities in blood coagulation and fibrinolysis are manifested as petechiae, ecchymoses, mucosal hemorrhages, and uncontrolled bleeding at venipuncture sites (2, 4–9); however, massive loss of blood is atypical and, when present, is largely restricted to the gastrointestinal tract. In fact, even in these cases, blood volume loss is insufficient to account for death. The presence of a maculopapular rash is a prominent feature (2, 4–9), but is not pathognomonic for EBOV HF. Fulminant EBOV infection typically evolves to shock, convulsions, and, in most cases, diffuse coagulopathy (2, 4–9). Death usually occurs 6–9 days after the onset of clinical symptoms.
Historical human clinical studies of EBOV HF have provided important descriptive information on the pathogenesis of these agents; the data are usually incomplete and occasionally contradictory or paradoxical. Here, we consider data obtained from these clinical studies and draw parallels focusing primarily on experimental infections of nonhuman primates. Rodent models are also available for EBOV HF; rodents are useful as a first screen for evaluating antiviral drugs and vaccine strategies, and genetically engineered mice clearly have utility for evaluating specific host-pathogen interactions. However, the disease course in rodents is far less faithful to the human condition than it is in nonhuman primate models (10). More specifically, mice do not exhibit the coagulation abnormalities that characterize primate EBOV infections (10, 11). The development of coagulopathy in EBOV-infected guinea pigs is uncertain, with findings varying among studies (10–12).

The selection of an appropriate nonhuman primate model for EBOV HF requires careful consideration of the species, sex, age, route of infection, dose of EBOV administered, and the nature of the challenge virus itself. Several primate species have been successfully employed to model ZEBOV HF, including African green monkeys (Chlorocebus aethiops), cynomolgus macaques (Macaca fascicularis), rhesus macaques (Macaca mulatta), and hamadryad baboons (Papio hamadryas) (reviewed in (13)). Few studies have evaluated the pathogenesis of SEBOV in nonhuman primates, thus, we will primarily focus on ZEBOV HF.

It is very difficult to compare results among the various published studies of ZEBOV HF in nonhuman primates because of considerable differences among the factors noted above. As an example, the challenge dose appears to have a profound effect on the course of disease in nonhuman primates. Cynomolgus macaques exposed by intramuscular injection with a low challenge dose of ZEBOV (10 plaque-forming units (pfu)) succumbed to infection 8–12 days after challenge (mean = 9.8 days), whereas cynomolgus monkeys exposed by intramuscular injection to a high dose (1000 pfu) of the exact same ZEBOV isolate died 5–8 days after challenge (mean = 6.3 days) (10, 14). Although challenge dose had a substantial effect on the course of disease in these animals, it did not change the sequence of events related to the development of clinical pathology or the immune response (T.W. Geisbert, unpublished observation). Another example of a variable that may cause disparity among previous studies is the species of nonhuman primate employed. Species-specific differences in the appearance of coagulopathies were reported in one study. Specifically, fibrin deposition in vessels was associated with ZEBOV infection of African green monkeys while hemorrhages were a feature associated with ZEBOV disease in baboons (15). However, there are a number of concerns with this study including the omission of histological stains for the detection of polymerized fibrin. Other studies have failed to detect species-specific differences in coagulopathy among different species of nonhuman primates (16).

As noted, EBOV infections are characterized by coagulation abnormalities that are generally consistent with disseminated intravascular coagulation (DIC) (4, 8). DIC is associated with both bleeding and thrombotic abnormalities, and, in fact, widespread thrombosis and bleeding commonly occur simultaneously. There are two major mechanisms that trigger DIC: 1) widespread injury to endothelial cells and; 2) release of tissue factor or thromboplastic substances into the circulation. Previously, it was reported that EBOV induces overexpression of tissue factor in primate monocytes and macrophages (16). While it is likely that tissue factor release from infected monocytes and macrophages plays a key role in inducing the development of the coagulation irregularities seen in EBOV HF, it also possible that other factors may contribute to the coagulopathy either indirectly by activating the tissue factor pathway through other mechanisms or by direct or indirect impairment of endothelial cell functions. In temporal studies using nonhuman primate models of EBOV HF, it appears that endothelial cells are not the primary cellular targets of EBOV (17). Based on these observations, any impairment of the endothelium during EBOV infection is likely the result of immune-mediated or other mechanisms that are independent of direct infection of endothelial cells. In this review, we focus on determining the role of the endothelium in contributing to the hemorrhagic diathesis of EBOV infection.

The endothelium as a direct target of EBOV

The vascular system is a multifaceted array of vessels that links the heart with assorted organs and tissues. In brief, the primary function of the vascular system is to serve as a barrier between the blood and interstitial compartments and to maintain homeostasis in response to various physiological or pathological changes. The lumens of blood vessels are lined by endothelial cells, which play a central role in mediating vascular tone, regulating cellular and nutrient trafficking, modulating coagulation, and sustaining blood fluidity. Cytokines, hormones, and neurotransmitters are involved in regulating endothelial cell functions. Endothelium has been postulated to play an important role in the pathogenesis of the coagulation disorders that characterize EBOV infections in primates (18, 19). However, the precise mechanisms have not been fully explained and the magnitude and overall contribution of viral replication in endothelial cells to the hemorrhagic diathesis is not completely understood and has been somewhat controversial.

Dysregulation of endothelial cell functions can cause a wide range of vascular effects that lead to changes in vascular permeability or hemorrhage (20, 21). Vascular damage can be induced by immunological mechanisms and/or by direct infection of the vascular tissue. Several microbial diseases are characterised by severe vascular lesions attributed to direct microbial-replication-induced damage to endothelial cells. For example, intracellular replication of Rickettsia rickettsii, the etiological agent of Rocky Mountain spotted fever, directly induces lethal injury to host endothelial cells, causing pathophysiological changes including thrombosis, hemorrhage, and vasculitis (22). Another example is Nipah virus infection, where a systemic vasculitis with extensive thrombosis is attributed to infection, damage, and necrosis of endothelial cells (23). The etiology of the hemorrhagic diatheses in fatal cases caused by the filoviruses MARV and EBOV was searched for in tissues from initial outbreaks in 1967 and 1976, respectively, but no vascular lesions were identified (24). Nonetheless, there has been much speculation that EBOV-replication-induced structural damage of endothelial cells triggers the hemorrhagic diathesis.
The subject of cytotoxicity mediated by the EBOV glycoprotein (GP) and its relevance in pathogenesis has been a topic of much debate. Yang and colleagues reported that GP expression caused cell death \textit{in vitro} and in primate vessel explants (18, 25), whereas subsequent work by other groups showed that most of the detached cells (>90%) were viable, suggesting that expression of GP interferes with cell attachment but does not trigger cell death (26, 27). Another group reported that the cytotoxic effect of GP \textit{in vitro} is associated with its level of expression. In this study, using a novel reverse genetics system, Volchkov and co-workers generated a mutant virus in which the editing site of the GP gene was removed (28). The authors noted that the mutant virus no longer produced sGP. Notably, the mutant was significantly more cytotoxic than wild-type virus, showing that cytotoxicity caused by GP is downregulated by EBOV through transcriptional RNA editing and expression of sGP. Thus, the authors postulated that transcriptional editing of the GP gene might therefore play an important role in EBOV pathogenesis by restricting cytotoxicity and thereby increasing viral loads and promoting spread. The apparent protective effect of transcriptional editing and sGP is consistent with results showing that EBOV replication does not directly induce cytolytic- or viral infection does not extensively disrupt the architecture of the vascular endothelium in ZEBOV-infected cynomolgus monkeys (17). While ZEBOV replicated in endothelial cells of these animals, endothelial cell infection was only seen focally at late stages of disease, after the onset of the hemorrhagic abnormalities that characterize EBOV HF (16, 36) (Fig. 1 and 2). Although ultrastructural evidence of endothelial cell activation and disruption was observed at midpoint to late stages of disease, it is likely that the vasoactive effects on endothelial cells are mediated indirectly as these changes were not associated with the presence of intracytoplasmic EBOV antigens (17). A smaller temporal study of ZEBOV infection of African green monkeys also showed that endothelial cell infection was associated with later stages of disease (15) so it appears that the dynamics of endothelial cell infection may be consistent among different species of primates.

Human and nonhuman primate endothelial cells are highly susceptible to EBOV infection \textit{in vitro} (17, 37–39). Evidence was first shown by Harcourt and colleagues who reported that EBOV inhibits the induction of genes by double-stranded RNA in human umbilical vein endothelial cells (37). It was subsequently shown by others that while ZEBOV is capable of replicating in microvascular and macrovascular human endothelial cells \textit{in vitro}, replication does not directly induce cytopathology (17).

It is interesting that while vascular endothelial cells are among the first cells in the host that come into contact with circu-
lating EBOV particles, they do not appear to be primary targets of EBOV in nonhuman primates. It is possible that the host factor required for efficient entry of EBOV is located on the basolateral surface and is thus somewhat protected from the initial viremia. Supporting this view is the finding that the first populations of EBOV-positive endothelial cells detected in cynomolgus monkeys were endothelial cells lining the hepatic sinusoids. As illustrated by Schnittler and Feldmann (40) in postulating a model for routes of filoviral dissemination, the portal liver sinususes are lined by a discontinuous endothelium that does not rest on a regular basement membrane. The endothelium contains transcellular gaps, allowing viral particles more immediate access to hepatocytes as proposed, but these gaps may also enhance access to the basolateral aspects of the sinusoidal endothelial cells. Not surprisingly, the adrenal gland is organized in a similar manner and endothelial cells lining sinusoids in the adrenal cortex are also earlier targets of EBOV in cynomolgus monkeys than other endothelial cell populations (17).

Although several species of nonhuman primates appear to faithfully reproduce human EBOV HF, clearly, there may be differences in susceptibility and response of endothelial cells to EBOV between humans and nonhuman primates. Four different types of cell-surface receptors have been proposed to play a role in EBOV entry including the C-type lectins, dendritic-cell-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN), and DC-SIGN-related (DC-SIGNR) factors (41–43). While DC-SIGN is expressed mainly on dendritic cells and some types of tissue macrophages, which are the primary targets of EBOV in humans and nonhuman primates, DC-SIGNR is expressed mainly on endothelial cells of the lymph nodes, liver, and placenta (44, 45). Importantly, Old World monkeys and apes have orthologues of human DC-SIGN; however, DC-SIGNR is purportedly missing in Old World monkeys but present in apes (46). Thus, if DC-SIGNR plays a major role in the ability of EBOV to efficiently enter endothelial cells, its absence on macaque endothelial cells versus presence on human endothelial cells could potentially contribute to observed differences in the frequency of EBOV-infected endothelial cells in target tissues. However, EBOV readily infects nonhuman primate endothelial cells in vitro (38, 39) so the overall significance of the difference in the expression of DC-SIGNR remains unknown.

The issue of direct EBOV infection of endothelial cells is further complicated by the diversity of endothelial cell subpopulations. For example, endothelial cells from different blood vessels and microvascular endothelial cells from different tissues were shown to have distinct and characteristic gene expression profiles (47). This raises the possibility that EBOV may have different affinities for different types of endothelial cells, and that the different types of endothelial cells may respond differently to EBOV infection and downstream events.

Mediators or factors that may affect vascular function during EBOV HF

Although direct interactions between endothelial cells and EBOV undoubtedly occur, soluble factors released from EBOV-infected monocytes, macrophages, and dendritic cells are thought to be key mediators in this process (36, 40, 48, 49). Serum levels of a variety of direct and indirect vasoactive factors are elevated in patients with EBOV HF and in experimentally infected nonhuman primates (Table 1).

The clinical manifestations of EBOV HF in nonhuman primates are in many ways consistent with clinical findings in severe sepsis. Similarities exist between the paradigm of inflammation in the pathogenesis of both sepsis and EBOV HF (49). The shared features include involvement of various cytokines and mediators such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α. Activation of the coagulation cascade is also prominent in both severe sepsis and EBOV infection of nonhuman primates as evidenced by increased D-dimer levels and decreased levels of circulating protein C in nearly all cases (16, 49).

Septic shock is characterized by a collapse of endothelial barrier function. The subsequent loss of fluid into the extravascular space results in severe and often life-threatening edema in the lungs, kidney, and brain of septic patients. Notably, permeability alterations of endothelia have been associated with plasma leakage produced by infection with several HF viruses such as dengue and Hantaan virus (50, 51). While edema has been noted in some cases of EBOV HF, it does not appear to be a prominent finding in EBOV-infected patients or in experimentally infected nonhuman primates. Thus, while EBOV infection either directly or indirectly may cause some change in endothelial barrier function, this change does not appear to be significant enough to manifest clinically as life-threatening edema. Clearly, there do appear to be perturbations of the endothelium associated with EBOV infections, as evidenced by decreased serum albumin levels noted in experimentally infected cynomolgus monkeys (17), and some ultrastructural evidence of activation (17), but these changes alone cannot account for the severity of EBOV HF. In recent years, the importance of the interaction between coagulation and inflammation as a response to severe infection has become increasingly appreciated. Inflammatory mediators upregulate procoagulant factors such as tissue factor, inhibit fibrinolytic activity, and downregulate natural anticoagulant pathways, in particular, the protein C anticoagulant pathway. Indeed, it ap-

### Table 1: The table lists cytokines, chemokines, and other mediators that may play a direct or indirect role in any alteration of endothelial cell function observed during EBOV infection in humans and nonhuman primates.

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Human EBOV HF</th>
<th>Nonhuman primate EBOV HF</th>
</tr>
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<tbody>
<tr>
<td>IFN-α</td>
<td>Increased [55]</td>
<td>Increased [33,57]</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Increased [55]</td>
<td>Increased [57]</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Not detected [56]</td>
<td>Not detected [33]</td>
</tr>
<tr>
<td>IL-6</td>
<td>Increased [56]</td>
<td>Increased [33,57,74]</td>
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<tr>
<td>IL-8</td>
<td>Not detected [56]</td>
<td>Increased [33,57]</td>
</tr>
<tr>
<td>IL-10</td>
<td>Increased [33,56]</td>
<td>Not detected [33,57]</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Not evaluated</td>
<td>Increased [33,74]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Increased [33,56]</td>
<td>Increased [33,57]</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Increased [36,62]</td>
<td>Increased [33]</td>
</tr>
<tr>
<td>Protein C</td>
<td>Not evaluated</td>
<td>Decreased [13]</td>
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<tr>
<td>Microparticles</td>
<td>Not evaluated</td>
<td>Increased [13]</td>
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pears that any impairment of the endothelium during EBOV infection is primarily due to release of vasoactive mediators or factors, such as nitric oxide (NO), from EBOV-infected monocytes, macrophages, and dendritic cells rather than being directly induced by the process of viral replication. Here, we briefly assess the potential of several mediators associated with EBOV infection of humans and nonhuman primates on endothelial cell structure and function.

Cytokines and chemokines
Cytokines are key mediators of inflammation and vascular dysfunction. They can induce changes in endothelial cell structure that affect permeability and they can also play a role in regulating the inflammatory response. Endothelial cells express a variety of cell-surface adhesion molecules in response to inflammatory stimuli that facilitate tethering, attachment, and transmigration of leukocytes. TNF-α has been shown in a number of studies to induce endothelial cell-surface changes. Notably, TNF-α can provoke acute pulmonary vascular endothelial cell injury in vivo and in vitro (52). TNF-α was found to act directly on cultured human vascular endothelium to induce a tissue factor-like procoagulant activity (53). It also causes reorganization of human vascular endothelial cell monolayers (54) and increases the permeability of endothelial cell monolayers by a mechanism involving regulatory G proteins (55). In combination with IL-1β, TNF-α prolonged endothelial cell dysfunction as measured in veins of human subjects (56). Furthermore, several studies have shown that anti-TNF-α treatment of diseases such as rheumatoid arthritis and anti-neutrophil cytoplasmic antibody-associated systemic vasculitis (AASV) improves endothelial function and endothelium-dependent vasomotor responses (57, 58).

Few studies have dissected the complex mediator-endothelial cell or immune cell-endothelial cell interactions, particularly with regard to the filoviruses. However, a seminal study by Feldmann and colleagues clearly shows that mediator-release from MARV-infected target cells can have deleterious effects on the integrity of the endothelium and may contribute to vascular instability (59). In these studies, the investigators showed that fluids from human monocyte/macrophage cultures infected with MARV increased the permeability of cultured human endothelial cell monolayers. The increase in endothelial permeability correlated temporally with TNF-α release, was inhibited by a TNF-α-specific monoclonal antibody, and the effect of TNF-α could be primed in the presence of agents such as H2O2. Such changes in endothelial permeability could ostensibly contribute to the hemostatic impairments noted during EBOV and MARV HF. Indeed, TNF-α likely plays some role in the vascular instability that occurs during EBOV HF. While increased plasma levels of TNF-α have been reported in nonhuman primates experimentally infected with EBOV, the increases were relatively small and were primarily associated with the later stages of disease (36, 60). However, as noted above, previous studies showed a priming effect for TNF-α in the presence of agents such as H2O2 (59). Because substantially increased systemic serum nitrate levels, indicating increased in vivo nitric oxide production, were observed in EBOV-infected humans and nonhuman primates (36, 60–62) the impact of low concentrations of TNF-α on vascular permeability and function cannot be discounted.

Other cytokines or chemokines may also be involved in modulating endothelial function during EBOV infections either directly or indirectly. For example, interferon (IFN)-α, IFN-γ, IL-6, and monocyte chemoattractant protein (MCP)-1 are upregulated during EBOV infection of humans and/or nonhuman primates (36, 60, 61, 63) and may have indirect effects on endothelial function. Increased mRNA transcripts of the chemokine IL-8 were detected in peripheral blood mononuclear cells of EBOV-infected nonhuman primates (36, 60). Notably, IL-8 was recently shown to contribute to dengue virus-induced modification of transendothelial permeability (64). As a final example, IL-10 is an anti-inflammatory cytokine that appears to be upregulated in human EBOV infections (61, 63), but may be diminished in EBOV infection of nonhuman primates (36, 60). While little is known about the role of IL-10 in vascular function, there is some evidence that IL-10 protects endothelial function after an inflammatory stimulus by limiting local increases in superoxide (65).

Nitric oxide (NO)
NO functions as both an autocrine and paracrine cellular mediator. In addition to its role as a vasodilator, NO inhibits platelet aggregation and decreases expression of proinflammatory molecules by the endothelium (6). Several lines of evidence suggest that the hyperproduction of NO by the inducible form of NOS (iNOS) may contribute to the hypotension, cardio depression, and vascular hyporeactivity in septic shock (67). Increased blood levels of NO were reported in nonhuman primates experimentally infected with EBOV (36, 60) and more recently in EBOV-infected patients (61, 62). Importantly, Sanchez and colleagues associated increased blood levels of NO with mortality (62). NO is known to have both protective and caustic effects and this autotoxic overproduction may represent the host’s endogenous counter-regulatory mechanism of protection against noxious agents, in this case EBOV. In general, microbes induce monocytes and macrophages to produce NO in attempt to control infection. However, in the case of EBOV, monocytes and macrophages are preferred target cells for viral replication. Enhanced replication in these cells may in turn exacerbate disease by producing large amounts of NO, resulting in deleterious effects. NO is an important mediator of hypotension (68) and as noted previously, hypotension is a prominent finding in EBOV HF (2).

Activated protein C (APC)
During microbial infection, activation of vascular endothelium by proinflammatory cytokines leads to hypotension, microvascular thrombosis, and organ damage. Recent studies advocate a link between coagulation and inflammation through the APC pathway. The protein C system is one of the main anticoagulant mechanisms in blood. Growing evidence suggests that protein C has direct anti-inflammatory properties and modulating activity on cellular functions, likely by blocking NF-κB nuclear translocation (69–71). A rapid decrease in plasma levels of protein C concomitant with progression of disease was noted in cynomolgus monkeys experimentally infected with EBOV (16). Several studies have shown that endothelial cell injury is prevented by APC (70, 72), thus, any impairment of the APC pathway would likely contribute to microvascular dysfunction, and ultimately to
the hypotension and multiple organ damage that typify EBOV infections.

**Microparticles**

Microparticles are small membrane vesicles with procoagulant and proinflammatory properties released from cells upon activation or during apoptosis (73, 74). Microparticles are derived from a variety of cells including platelets, lymphocytes, granulocytes, monocytes, and endothelial cells. While microparticles are found in the circulation of healthy subjects, elevated numbers have been associated with a variety of diseases with vascular involvement and coagulation disorders including DIC and systemic inflammatory disease. Importantly, temporal increases in the numbers of circulating microparticles were detected during EBOV infection of cynomolgus and rhesus monkeys (16). Many of these microparticles expressed tissue factor, indicating that they may be derived from monocytes (16); however, it is likely that other populations of cells also contributed to the increased numbers of microparticles seen in the EBOV-infected macaques. Notably, recent studies have shown that shed microparticles from T lymphocytes impaired endothelial function and regulated endothelial protein expression (35). Because extensive apoptosis of bystander T lymphocytes appears to be a prominent feature of EBOV infection of humans and nonhuman primates (36, 76–78), it is plausible that large numbers of T lymphocyte-derived microparticles may also contribute to the observed vascular and coagulation complications that develop during EBOV infection of primates.

**Conclusions**

Recent studies suggest that while primate endothelial cells are permissive to EBOV entry and replication, EBOV replication-induced cytolysis of endothelial cells is not the major trigger for the coagulation abnormalities that characterize EBOV HF (17). These studies have shown that the coagulation disorders in experimentally infected nonhuman primates are largely caused by activation of the tissue factor pathway (16, 79). Moreover, EBOV-infected monocytes and macrophages are thought to be key sources for tissue factor protein during infection, although, subendothelial structures are also potential sources of tissue factor. Subendothelial tissue factor is constitutively present in fibroblasts, pericytes, and macrophages and could play a secondary role in the developing coagulopathy if exposed to blood components subsequent to permeability changes or damage to the endothelium. In addition, activation of the endothelium by any number of stimuli can further amplify the procoagulant activity of monocytes as it has been shown that adhesion of monocytes to activated endothelium induces tissue factor on monocytes (80).

The importance of tissue factor in playing a key role in triggering the coagulation abnormalities that typify Ebola infections is supported by a recent in vivo study. Indeed, treating EBOV-infected rhesus monkeys with recombinant hematode anticoagulant protein c2 (rNAPc2), a potent inhibitor of tissue factor-initiated blood coagulation, significantly improved survival of the infected animals (79). Survival of the rNAPc2-treated macaques was associated with reduced activation of coagulation and fibrinolysis, and also by attenuation of the systemic proinflammatory response as evidenced by lower levels of IL-6 and MCP-1. These results demonstrate that cross-talk between inflammation and coagulation appears to play a central role in the development of EBOV HF (Fig. 3). Activations of coagulation and inflammation during severe infections, such as EBOV HF, appear to be linked bimodally. Cytokines and chemokines play an important role in the procoagulant state that follows severe infection. Indeed, it is known that IL-6, MCP-1, and TNF-α can induce the release of tissue factor from various cells (81–84) contributing to the dysregulation of the coagulation system. There is also solid evidence that activated coagulation factors or products in turn are capable of eliciting a proinflammatory response. For example, D-dimers and fibrin fragment E induce synthesis and release of IL-6 from monocytes and macrophages (85, 86). Thrombin was shown to stimulate the release of proinflammatory cytokines and chemokines including IL-6, IL-8, and MCP-1 by monocytes and endothelial cells (87–90) likely through the activation of protease-activated receptors (91). Thus, it appears that many of these processes are complex, interrelated, and tightly regulated.

A key event in the initiation, localization, and dissemination of vascular injury involves activation of the vascular endothelium by a variety of stimuli including cytokines and other host cell factors as discussed in this review, complement split products, EBOV GP, and related interactions between endothelial cells and various leukocytes. The role of complement in contributing to the coagulopathy is unknown and warrants systematic investigation. Activation of the complement system can result in exposure of membrane surfaces capable of amplifying the initial tissue factor stimulus by promoting the assembly of the factor VIIIa-factor IXa and the factor Xa-factor Va complexes. EBOV produces four soluble GP during infections in addition to the full-length GP found on the surface of mature virions (2,92). Recent studies have shown that the full-length GP observed on the surface of virions is capable of activating human macro-

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**Figure 3: Model of EBOV pathogenesis in primates.** EBOV infection of monocytes, macrophages, and dendritic cells appears to be central to the development of disease. Infection and/or interaction of these cells with EBOV stimulates the release of a variety of mediators and factors that trigger a host of downstream events including activation of the coagulation system and disruption of the vascular endothelium. Symbols: black bar, platelets; ovals, red blood cells.
phages while the secreted GP did not appear to play any role in activation of these cells (93). It will be important to determine whether endothelial cells behave in a similar manner when exposed to these EBOV glycoproteins.

In summary, we are only beginning to understand how coagulation abnormalities develop during EBOV HF. A particular obstacle has been the fact that human cases of EBOV HF occur in remote geographic locales where it is not logistically possible to carefully monitor patients with modern technologies. Although several species of nonhuman primates appear to faithfully reproduce human disease, technologies to adequately measure important pathophysiological parameters including blood pressure and endothelial function have up to this point been impracticable in the Biosafety level 4 facilities that are required to work with EBOV. However, increased concern about the natural or unnatural introductions of agents including EBOV has driven increased investment in basic research and construction of a network of biocontainment laboratories. This development coupled with recent innovations in implantable bio-telemetry technology now make it possible to follow in detail multiple clinical parameters during the course of an infection in animal models including nonhuman primates. Telemetry systems are available for continuously monitoring multiple systems including blood pressure, respiration, electrocardiogram, electroencephalogram, and core temperature. Indeed, advanced systems will be able to expand data collection to include the above parameters plus ventricular blood flow, and inrathoracic pressure.

Clearly, an increased understanding of the mechanisms of EBOV pathogenesis will augment our ability to develop effective countermeasures against this notoriously aggressive pathogen.

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Abbreviations

EBOV, Ebola virus; HF, hemorrhagic fever, IFN, interferon; IL, interleukin; MCP, monocyte chemotactic protein; NO, nitric oxide; ROS, reactive oxygen species; TF, tissue factor; TNF, tumor necrosis factor

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