Ebola Hemorrhagic Fever and Septic Shock

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Ebola virus is the cause of sporadic outbreaks of lethal Ebola hemorrhagic fever (EHF) in central Africa. Despite the difficulties of studying this virus, much has been learned over the past decade about the pathogenesis of Ebola virus infection in humans and nonhuman primates. Two articles in this issue of the *Journal of Infectious Diseases* report further progress. The article by Bosio et al. [1] confirms findings that the virus is able to infect dendritic cells (DCs), impairing their innate antiviral activity and limiting their ability to initiate adaptive immune responses [2]. The article by Geisbert et al. [3] identifies a molecular trigger for disseminated intravascular coagulation (DIC) through the expression of tissue factor (TF) on the surface of virus-infected monocytes and macrophages. Together, these findings shed light on early events that both permit rapid viral dissemination and cause some of the major features of EHF.

Geisbert et al. [3] note that TF expression by monocytes and macrophages has also been identified as the basis of the DIC that is seen in septic shock and point out that 2 other features of EHF, high levels of circulating proinflammatory cytokines and lymphocyte apoptosis, also occur in severe bacterial infections. Other investigators have also noticed these similarities and have suggested that the comparison of EHF with septic shock could lead to insights into pathogenesis and to improvements in therapy [4]. Although viral and bacterial infections obviously differ in fundamental respects, it is now recognized that the interactions of pathogens or their components with pattern-recognition receptors on macrophages and related cells tend to evoke fairly similar sets of innate responses. It is therefore worth comparing EHF with septic shock to see to what extent they share basic mechanisms of pathogenesis.

To do this, it is first necessary to describe the basic features of each syndrome. The limited published information on human Ebola virus infection has almost all been obtained during recent outbreaks caused by the Zaire subtype, in which reported case-fatality rates have ranged from ~60% to 90% [5–7]. High fever, prostration, and a variety of nonspecific signs and symptoms begin about a week after exposure to the virus, often leading to steadily worsening coagulopathy, shock, and death during the second week of illness. A maculopapular rash may be noted early in the course of disease, but the clinical picture otherwise lacks specific diagnostic features. For this reason, the first individuals to become ill usually are misdiagnosed as suffering from bacterial infection, yellow fever, or malaria. Hemorrhagic manifestations tend to be limited to petechiae, ecchymoses, oozing from venipuncture sites, hematuria, and melena. Limited data on immune responses during EHF were obtained during the 1995 Kikwit epidemic, and more-extensive data were obtained during 2 outbreaks in Gabon [8–12]. Analysis of blood samples has shown high levels of proinflammatory cytokines, including tumor necrosis factor (TNF)–α, and evidence of intravascular apoptosis. Host responses that appear to be predictive of survival or death are described below.

Our most detailed knowledge of Ebola virus pathogenesis has been obtained through laboratory studies of nonhuman primates, in which inoculation, by various routes, with the Zaire virus causes rapidly progressive infection, ending in death 6–8 days after challenge [13–15]. The virus is able to replicate in a broad range of cells and causes their lysis. Macrophages are major early targets of infection and appear to be the major source of proinflammatory cytokines [16]. The virus disseminates rapidly to fixed and mobile mononuclear phagocytic cells in the liver, spleen, and other tissues, then spreads to hepatocytes, fibroblasts, and parenchymal cells of other organs. Infection causes extensive necrosis in the liver, spleen, and lymphoid tissues but does not appear to result in major injury to parenchymal cells of the heart, lungs, or kidneys. Lymphocytes are not infected but nevertheless undergo

massive apoptosis during the later stages of illness. DIC leads to extensive microvascular thrombosis and widespread fibrin deposition. Some investigators have attributed the DIC to infection of the endothelial lining of blood vessels [17, 18], but Geisbert et al. [3] have found little evidence of viral replication in these cells before the late stage of illness.

The many types of bacterial infection and the great variety of circumstances in which septic shock can develop make it difficult to summarize the syndrome; the reader is referred to recent reviews for more information [19, 20]. Most studies have focused on infection with gram-negative bacteria. With some exceptions, such as meningococcemia, systemic illness tends to occur in a setting of trauma, chronic disease, or underlying immunodeficiency. The first signs of bacterial sepsis include fever, chills, malaise, and change in mental status, symptoms that may progress, over the course of a few days, to hypotension and reductions in renal, hepatic, pulmonary, and/or cardiac function. DIC develops, but, as in the case of EHF, severe hemorrhage is infrequent. Lymphocyte apoptosis may occur in severely ill patients [21]. With the exception of a primary focus of infection, such as an area of trauma, there is usually little tissue destruction, and dysfunctional organs can return to their previous state once the infection is brought under control through antibiotic therapy and other measures [19].

The major clinical features of septic shock are brought about indirectly, through the action of cytokines, chemokines, and other mediators produced by monocytes, macrophages, and related cells as a result of the binding of lipopolysaccharide (LPS) and other bacterial components to pattern-recognition receptors, including Toll-like receptors. Additional mediators are released by neutrophils and other cells recruited to the site of infection. These substances produce the familiar features of a local inflammatory response. Proinflammatory cytokines (e.g., interleukin [IL]–1β, IL-6, and TNF-α) and other mediators (e.g., nitric oxide, reactive oxygen molecules, and

leukotrienes) cause vasodilatation of local blood vessels, increase the permeability of the endothelial lining, up-regulate the expression of endothelial surface-adhesion molecules, initiate coagulation, and activate immune cells, triggering the release of additional cytokines. If the infection continues to spread, the accumulation and systemic distribution of increasing quantities of these mediators produces fever, a generalized increase in vascular permeability, loss of vascular smooth-muscle tone, hypotension, DIC, and multiple organ dysfunction—that is, the syndrome known as septic shock. The proinflammatory response is also accompanied by the release of a variety of "anti-inflammatory" mediators, such as IL-4, IL-10, and soluble receptors for IL-1β and TNF-α [19].

Many features of septic shock can be replicated by injecting animals with LPS, TNF-α, or other proinflammatory cytokines. However, efforts to treat patients with sepsis by blocking these mediators have either failed to provide benefit or have resulted in increased mortality. This has led to the realization that septic shock involves an extremely complex network of actions and counteractions and that attempts to manipulate it may produce unexpected results. These observations also suggest that proinflammatory cytokines may be helping to clear infection even while causing adverse systemic effects, so that their inactivation results in progression of disease.

To what extent does EHF resemble septic shock? The above descriptions indicate that direct tissue injury, especially the destruction of macrophages and related cells, plays a more significant role in EHF than in most cases of gram-negative bacterial infection. However, they also suggest that several major features of the 2 syndromes—such as refractory hypotension, DIC, and lymphocyte apoptosis—are brought about indirectly, by host responses to infection.

The difficulty of performing clinical studies during outbreaks of Ebola virus infection has provided little opportunity to study the etiology of the hypotension as-

sociated with the disease, but laboratory studies suggest that it has a similar basis to that of septic shock. Mediators that cause loss of vascular smooth-muscle tone, such as nitric oxide, have been detected in Ebola virus–infected nonhuman primates. Similarly, studies of the related Marburg virus have shown that cytokines released by infected macrophages, especially TNF-α, cause increased endothelial permeability, an effect that the investigators compared to that which occurs in septic shock [22, 23]. One alternative explanation for the vascular dysfunction in EHF is direct viral infection of the lining of blood vessels. However, although Ebola virus replicates in endothelial cells in culture, studies have revealed only limited infection of these cells in Ebola virus–infected nonhuman primates during the late stages of disease, as noted above. It has also been proposed that the virion surface glycoprotein or its secreted variant may cause direct endothelial injury [24, 25]. However, as in the case of septic shock, the effects of circulating proinflammatory cytokines and other mediators appear to be a sufficient explanation of the vascular dysfunction seen in EHF.

The expression of TF by stimulated monocytes was first demonstrated nearly 30 years ago, and a role for leukocyte-derived TF in causing the coagulopathy in EHF was proposed in 1995 [26, 27]. In bacterial sepsis, TF production is induced through the activation of the NF-κB and activator protein–1 pathways by LPS, TNF-α, and a variety of other mediators and is potentiated through the action of platelets and granulocytes [28–30]. Blood clotting is initiated on the surface of the activated macrophage through the binding of TF to factor VIIa. As described by Geisbert et al. [3], this results in the formation of a fibrin meshwork over infected cells, perhaps limiting further viral dissemination. The release of TF-bearing membrane microparticles may play a major role in inducing DIC. As in the case of septic shock, the progression of infection is accompanied by a decrease in levels of activated protein C in plasma [30]. In

EHF, blood clotting can be induced both by direct viral infection of monocytes/macrophages and by cytokine effects on the same cell population, perhaps explaining the severity of the coagulopathy. The finding that endothelial cells of Ebola virus–infected monkeys express little or no TF confirms the central role of the macrophage in the induction of EHF syndrome.

Lymphocyte apoptosis appears to be another feature of EHF and septic shock that is brought about by host responses. Massive lymphocyte apoptosis has been demonstrated in Ebola virus–infected nonhuman primates [18]. In the outbreaks in Gabon, blood samples from infected patients who died showed markers of apoptosis, such as Fas, Fas ligand, and decreased plasma mRNA of the antiapoptotic protein Bcl-2 [9, 31]. It is not known whether the apoptosis in EHF is restricted to T cells or if it affects both T and B cells. In septic shock, apoptotic depletion of lymphocytes has been reported in both animals and humans [32]. In an animal model, caspase-3 inhibitors reversed lymphocyte apoptosis and improved survival, as did the adoptive transfer of T cells overexpressing Bcl-2 [33, 34]. At present, only lymphocytes are known to undergo apoptosis in EHF, but, in septic shock, apoptotic cell death is also seen in the gastrointestinal and bronchial epithelium [32]. Further characterization of the apoptotic pathways in these syndromes may help clarify the role that this process plays in progression of disease.

Defective immune responses appear to play an important role in both EHF and septic shock, but the mechanisms differ significantly in the 2 syndromes. One difference between the 2 syndromes is reflected in susceptibility to infection. Septic shock usually occurs in a setting of trauma or impaired immune function, and the progression of infection is accompanied by increasing immune dysfunction, as the release of anti-inflammatory cytokines and soluble receptors and the loss of lymphocytes and other immune cells lead to a state of anergy [19]. In contrast, EHF can develop in a healthy, im-

munocompetent host. The inoculation of even a few Ebola virions into a nonhuman primate results in rapidly overwhelming infection, and most human cases of EHF also begin, presumably, in a setting of normal immune function. Such vulnerability may be caused, in part, by a lack of antigen-specific immunity to Ebola virus, but a more important factor appears to be the pathogen's ability to suppress innate and adaptive immune responses, permitting rapid dissemination. Such viral countermeasures presumably play their most important role by permitting spread of virus during the early, presymptomatic phase of infection, and their existence may therefore not be reflected in levels of circulating cytokines measured after the onset of illness. The possibility that some humans may be able to resist Ebola virus–induced immunosuppression and clear the agent without becoming ill is discussed below.

Massive infection of macrophages and related cells by Ebola virus suggests that it is able to block or evade the cells' innate antiviral mechanisms. Animal experiments and in vitro studies using cultured primary human macrophages have shown that infection induces the release of proinflammatory cytokines and chemokines [16, 17]. However, several investigators have found that the virus inhibits the synthesis and release of type I interferon (IFN) [35–38]. The viral protein VP35 has recently been shown to inhibit the activation of IFN regulatory factor 3, which is required for transcription of a number of genes involved in the type I IFN response [37, 38]. However, published reports are not unanimous in describing such virus-induced suppression; for example, Hensley et al. [17] have identified early IFN-α production in Ebola virus–infected human macrophages and nonhuman primates.

In addition to macrophages, DCs also play a critical role in the clearance of viral infection because they are the cells that most efficiently activate naive T cells to initiate antigen-specific immune responses [39]. Studies from recent out-

breaks of Ebola virus showed an association between a persons's surviving Ebola virus infection and the early appearance of anti–Ebola IgM and IgG, a phenomenon that is consistent with the need for effective DC function [9–12]. Ebola virus infection selectively inhibits the secretion of TNF-α, IL-1β, and IL-6 by monocyte-derived DCs and impairs their ability to activate naive T cells [1, 2]. Hensley et al. [17] have also reported that Ebola virus–infected monocyte-derived DCs do not release proinflammatory cytokines, but, in contrast to the authors of the former 2 studies [1, 2], they detected the release of IFN-α. Because plasmacytoid DCs are more important sources of IFN-α in immune responses than are monocyte-derived cells, further studies should compare the effect of Ebola virus infection on cells of both subsets [40].

A unifying concept in the study of EHF and septic shock is that early events play a critical role in determining the outcome of infection. Virus-induced suppression of innate and adaptive immune responses may be critical in permitting rapid viral dissemination in EHF. In the case of bacterial infection, it is also likely that events during the early, asymptomatic phase of infection determine whether a bacterial "challenge" results in clearance of the organism or progression to systemic disease. The likelihood that an individual will develop septic shock may, to some extent, be determined by inherited variation in cytokine responses, as reflected in the timing of mediator release and the relative amounts of pro- and anti-inflammatory cytokines [19, 41]. Some researchers have argued that, because host innate responses play their most important role in preventing systemic disease, their function cannot be understood solely on the basis of data obtained after the onset of clinical illness [41].

The critical role of early events in the outcome of Ebola virus infection appears to be confirmed in studies of immune responses in infected patients who died and in those who survived 2 EHF epidem-

ics in Gabon; in 1 of the epidemics, almost all the patients were infected more or less simultaneously [9, 10]. Survivors were characterized by the presence of IL-1β, IL-6, TNF-α, and anti–Ebola IgG in blood samples obtained early in the clinical course. In patients who died, however, these cytokines tended to appear later and were accompanied by IL-10 and soluble receptors for IL-1β and TNF-α, and antibody responses were not detected. Of interest, an elevated level of IL-10 in the presence of TNF-α has also been reported to be predictive of death in septic shock [42]. These data suggest that early inflammatory responses can limit the extent of infection and help bring about a successful transition to antigen-specific immunity. Interestingly, although IFN-α was detected in blood samples from patients with Ebola virus during the 1995 outbreak and has been demonstrated in samples from infected nonhuman primates that died, it was not found in samples from patients in the outbreaks in Gabon [9, 10, 43, 44].

Taken to its logical conclusion, the notion that effective, early innate responses can prevent the development of systemic disease predicts that some humans infected with Ebola virus may be able to fight off the virus without becoming ill. Leroy et al. have, in fact, found that some persons in close contact with Ebola virus–infected patients developed brisk elevations of proinflammatory cytokines, had viral genetic material (but no infectious virus) in blood samples, and developed anti–Ebola virus antibodies, suggesting that they underwent asymptomatic infection [43, 44]. Their reports suggest a "bimodal" model of infection, similar to that described above for bacterial sepsis, in which a pathogen either is successfully cleared by early host responses without causing disease or else escapes these mechanisms and produces systemic illness. Further studies will be required to confirm these findings.

The comparison of EHF with septic shock suggests that their most important shared feature is the central role of the macrophage in inducing some of the major features of illness. Further progress in the understanding of host-pathogen relationships during the early phase of infection may lead to the discovery of interventions that can prevent the development of disease. The treatment of full-blown EHF or septic shock is a much more difficult problem, since the complex network of host responses evoked by both Ebola virus infection and gram-negative bacterial infections may present a major barrier to effective therapy. However, recent success in reducing mortality from septic shock through treatment with recombinant activated protein C suggests that progress in treating EHF may also be possible [45]. Complete success is unlikely to be achieved until an equivalent of antibiotic therapy has been devised for Ebola virus infection.

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