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Ebola Virus: Immune Mechanisms of Protection and Vaccine Development

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Vaccination is one of our most powerful antiviral strategies. Despite the emergence of deadly viruses such as Ebola virus, vaccination efforts have focused mainly on childhood communicable diseases. Although Ebola virus was once believed to be limited to isolated outbreaks in distant lands, forces of globalization potentiate outbreaks anywhere in the world through incidental transmission. Moreover, since this virus has already been transformed into weapon-grade material, the potential exists for it to be used as a biological weapon with catastrophic consequences for any population vulnerable to attack. Ebola hemorrhagic fever (EHF) is a syndrome that can rapidly lead to death within days of symptom onset. The disease directly affects the immune system and vascular bed, with correspondingly high mortality rates. Patients with severe disease produce dangerously high levels of inflammatory cytokines, which destroy normal tissue and microcirculation, leading to profound capillary leakage, renal failure, and disseminated intravascular coagulation. Vaccine development has been fraught with obstacles, primarily of a biosafety nature. Case reports of acutely ill patients with EHF showing improvement with the transfusion of convalescent plasma are at odds with animal studies demonstrating further viral replication with the same treatment. Using mRNA extracted from bone marrow of Ebola survivors, human monoclonal antibodies against Ebola virus surface protein have been experimentally produced and now raise the hope for the development of a safe vaccine.

Keywords: *Ebola virus; immune system; vaccine development*

Ebola virus is a filovirus, a group of viruses that induce hemorrhagic fever. Although the natural reservoir of Ebola remains unknown, researchers believe the virus to be animal borne and native to Africa (Centers for Disease Control and Prevention 2002). Humans become infected by a process that researchers do not yet understand, but it likely includes incidental exposure to infected animals (Centers for Disease Control and Prevention 2002). Although human outbreaks of Ebola hemorrhagic fever (EHF) have occurred only in Africa, this in no way minimizes the potential threat to other countries given the relative ease and frequency of air travel from this endemic area. Moreover, the history of biological weapon development suggests that a variety of hemorrhagic fever viruses have, at the very least, been a significant component of biological weapon research and that in some countries such as the former Soviet Union, these

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viruses were successfully weaponized (Center for Nonproliferation Studies 2000). Although the former Soviet Union joined the United States in 1992 to bring an end to their biological weapons program, the statuses of other state-sponsored programs are unknown (Center for Nonproliferation Studies 2000). The bioterroristic events of 2001 involving another deadly pathogen, anthrax, remind us that outbreaks of unusual infections must now be viewed through a different lens and that the development of effective treatments and vaccines must be significant priorities.

Although surveillance, hygiene, and barrier methods remain top priorities in isolation and containment of this deadly virus, vaccine development would be of great benefit to the local population and the scientists who study this virus. Although the development of a vaccine against Ebola virus continues to be promising, there are substantial obstacles. This article presents a review of the immunologic pathogenesis and diagnosis of Ebola virus, a brief historical profile of vaccine development, and new approaches to the development of an effective Ebola vaccine.

Immunologic Pathogenesis and Diagnosis of Ebola Virus

As discussed in the article by Casillas and others in this issue, a number of tests, including viral antigen assays, viral isolation, and IgM/IgG antibody assays (ELISA), have proven to be useful biomarkers for Ebola virus identification and progression of disease. Evidence suggests that successful recovery from Ebola virus is dependent on an effective and tightly regulated immune response. Fatal cases are marked by incomplete and unsustained T-cell responses (Nabel 1999). Moreover, exceedingly high levels of inflammatory cytokines (IL-2, IL-10, IFN-γ, TNF-α, and IFN-α), which are produced in infected macrophages, have been found among fatal EHF cases. These high levels of inflammatory cytokines correlate with disease severity and are associated with severe capillary leakage, microvascular damage, and activation of the clotting cascade (Feldmann and others 1999; Villinger and others 1999; Borio and others 2002). In addition, IL-6 levels are unusually low among fatal cases, suggesting that the likely source of IL-6, endothelial cells, are completely disabled by viral replication (Villinger and others 1999).

Antigen-capture enzyme-linked immunosorbent assay (ELISA) for IgM and IgG antibodies to Ebola virus is a sensitive diagnostic tool that is particularly useful if patients can be identified early in the course of the disease. Because IgM antibodies to Ebola virus appear 2 to 9 days after infection, the acute phase (IgM) response may not be easily detected even with ELISA technology if early symptoms are dismissed by patients. Since the appearance of IgG antibodies can take upward of 20 days from symptom onset, this marker becomes detectable only with onset of recovery and thus may also have limited value (Borio and others 2002). Viral antigen detection by ELISA and reverse transcriptase–polymerase chain reaction are particularly useful tests that are rapid and sensitive. Unfortunately, they are limited by the fact that antigen positivity in the serum generally disappears 7 to 16 days after symptom onset; in contrast, antigen positivity in seminal fluid can be detected for up to 3 months (Rowe and others 1999). Investigations are currently under way regarding the totality of immunological response to Ebola virus. This ongoing work should provide the necessary foundation for further refinement of the best markers for diagnosis as well as for vaccine development.

Immunological Manifestations among Survivors of EHF

EHF is a relatively rare but devastating viral infection associated with high mortality. As seen in Table 1, a brief incubation period of 2 to 21 days (average of 7) is followed by an abrupt onset of nonspecific immunologically mediated symptoms such as fever, muscle and joint pain, and headache, all of which could easily lead clinicians to diagnose other systemic infections (Borio and others 2002). This typical constellation of symptoms is attributed to the acute phase response. The ensuing clinical manifestations are associated with the release of proinflammatory cytokines such as TNF-α, IL-2, and the interferons (Janeway and others 1999). By the time hemorrhagic features such as hemorrhagic rash, bloody diarrhea, or hemoptysis occur, the infection is well established. Oddly enough, the proinflammatory cytokine IL-6 is elevated among EHF patients. Villinger (1999) pointed out that this elevation is consistent with the observation that Ebola virus invades the endothelial cells, thereby preempting

Table 1. Trajectory of Ebola Hemorrhagic Fever

Mode of Transmission	Incubation	Early Phase Signs and Symptoms (2-7 days)	Late Phase Signs and Symptoms (7-14 days)
Person to person (bodily fluids) Infected nonhuman primates occasionally provide transmission Aerosol transmission suspected from infected monkeys	2-21 days	Fever Extreme fatigue Diarrhea, nausea, and vomiting Abdominal pain Anorexia Headache Arthralgias/myalgias	Hemorrhagic rash Tachypnea Confusion, somnolence Hearing loss Other hemorrhagic signs, which may include epistaxis, mucosal bleeding (gums), hematuria, hemoptysis, hematemesis, melena, conjunctival hemorrhage

SOURCE: Bwaka and others (1999); Peters and others (1999); Sadek and others (1999); Streether and others (1999); Wilson and others (2001); Borio and others (2002); Centers for Disease Control and Prevention (2002).

cytokine production, in particular IL-6. The overwhelming production of the interferons likely contributes to the capillary leak syndrome and hemorrhagic rash associated with EHF. Individuals who succumb to EHF develop these clinical features early on (Streether 1999), which suggests that any weakness of the immune system is additional risk for mortality. In fact, the only predictor affecting survival from EHF that Sadek and others (1999) found was advancing age, a variable commonly associated with immune system decline. For example, the case fatality of persons older than 59 years was 95% as compared to a rate of 77% among individuals younger than 15 years. Other factors include the individual's inherent immunogenetic makeup. Ksiazek and others (1999) found that many patients who died from EHF during the 1995 Kikwit epidemic had higher Ebola antigen and virus titer levels irrespective of age. Moreover, many of those who died failed to produce any detectable antibody (Ksiazek and others 1999). The fact that those who died experienced the same level of exposure to the virus as those who survived suggests at least the possibility of an inherent immunodeficiency.

Among EHF survivors, arthralgias or myalgias are common (Rowe and others 1999). In a study of 20 Ebola survivors, many other symptoms that can persist during convalescence, such as abdominal pain, extreme fatigue, and anorexia, appear to resolve by 21 months. In contrast, almost two-thirds of the survivors continued to suffer from arthralgias or myalgias, and, in many instances, these constituted significant health problems. Symmetric polyarthropathies are associated with other viral infections, such as hepatitis B, rubella, and parvovirus (Schnitzer and Penmetcha 1996). These arthropathies result from either viral replication or the deposition of immune complexes in joint tissue. In other studies, antibody levels were greater among convalescents with arthralgias, indicating (indirectly) the role of persistent low-level antigenic stimulation (Dowell and others 1999; Rodriguez and others 1999; Roels and others 1999; Rowe and others 1999).

The most accurate test currently available to measure viral antigen uses ELISA and reverse transcriptase polymerase. However, antigen positivity generally disappears 7 to 16 days after symptom onset, so even the most sensitive tests have limitations. Case reports of persistent antigen thriving in seminal fluid for up to 3 months (Rowe and others 1999) suggest that the virus may continue to be present, although it is undetectable in serum. Ongoing serial antibody measurements in patients with persistent arthralgias may be helpful in gauging recovery.

Historical Profile of Vaccine Development

Historically, vaccines were developed by microbiologists who concentrated on identifying and isolating the pathogenic organism and growing it in quantity in a laboratory. Such pathogens were then treated with formaldehyde, radiation, or other means of killing the organism, which could then be safely introduced to humans or animals in sufficient quantity and on an adequate schedule to elicit an immune response that would protect against clinical disease. In many in-

stances, only some of the immunogenetic portions (antigens) of the organism were sufficient to elicit protection against the intact pathogenic organism (Wilson and others 2001).

Many vaccines do not prevent infection but instead limit the degree of infectivity to a level insufficient to cause clinical manifestations. When infection occurs, the development of pathologic alterations is limited due to the strong memory response generated by the vaccine. Such is the case with polio vaccines. They do not prevent infection but do prevent a clinical disease.

The approaches to vaccine preparation used in the past century were satisfactory in many circumstances. On the other hand, where the pathogen changes by mutation, as happens with the flu viruses and with HIV, such a static approach may not be effective. New vaccine development has entered into a realm, only partially understood, that relates to key aspects of immunology, such as the presentation of antigen, response to antigen and factors determining the dimensions and qualities of immune responses, and the time required to develop such responses. Furthermore, the molecules on the cell surface responsible for recognizing foreign materials are under the control of major immunogenetic systems of the body (those of the histocompatibility locus genes). Advances in these areas are being incorporated into the development of vaccines against Ebola virus.

Information about the type of immunity that is protective in individuals with mild infections, or even those who are exposed but do not develop clinical infections, is needed. The questions center on the need for antibody immunity (and what type of antibody immunity—mucosal or systemic) and on whether cell-mediated immunity is a critical element in protection against clinical infections. Both humoral and cellular immunity are detected in Ebola survivors, but their relative contribution to protection is not known.

Ebola Vaccine Development

Ebola vaccine development has been fraught with obstacles. Identification of the causative agent and a capacity to grow the virus in the laboratory is requisite for vaccine development. Because of its virulence, Ebola virus must be handled under rigorous Biosafety Level 4 (BSL-4) conditions, which require expensive special facilities. Currently, there are only 2 BSL-4 laboratories for Ebola virus in the United States. More important, a vaccine that consists of an attenuated (weakened) or even killed virus of this virulence is unlikely to be accepted by national health agencies or the public for safety reasons.

Currently, the use of convalescent plasma for passive protection against Ebola remains controversial because such treatment may contain live virus (World Health Organization 1997). A number of studies using animal models, including monkeys, have shown some positive results using hyperimmune sera for treatment of Ebola infection. No controlled studies have been done to demonstrate the effectiveness of hyperimmune serum in humans. Reports showing improvement of acute Ebola infection in patients following the transfusion of convalescent plasma (Mupapa and others 1999; Borio and others 2002) are at odds with research studies demonstrating viral replication in experimentally infected animals (Jahrling and others 1999).

Despite the drawbacks, recent work on developing an effective filovirus vaccine that stimulates a broad immune response remains promising (Hevey and others 1998; Xu and others 1998), and other avenues of vaccine preparation are being explored (Folks 1998; Sullivan and others 2000). Human monoclonal antibodies against Ebola virus surface protein have been experimentally produced using mRNA extracted from bone marrow of Ebola survivors. This approach presents the possibility of a safe therapy (Peters and others 1996; Maruyama, Parren, and others 1999; Maruyama, Rodriguez, and others 1999). In this line of molecular gene research, it was found that the DNA coding specific microbial and viral antigens could be isolated so that the whole organism need not be used for immunization. Furthermore, the DNA (genes) could be transferred to nontoxic organisms and introduced into the body to excite immune responses.

Newer methods of vaccine development are focusing on isolating the genes that code for specific viral proteins. These genes can be introduced by injection and will function to produce only one (or a few) specific, but harmless, proteins of the virus (Xu and others 1998). These, in turn, will stimulate immunity. Ebola virus is a large RNA virus that has 7 genes that encode 8 proteins, including a single glycoprotein coat molecule (Folks 1998). Specific components of the Ebola virus, including the glycoprotein coat and a nucleoprotein, have been shown to induce protective

immunity in guinea pigs and primates (Burton and Parron 2000). When injected into an animal or person, the nucleic acid portions of viruses induce cell-mediated immunity (Wilson and Hart 2001). Cell-mediated immunity can be effective in controlling microorganisms (Vanderzanden and others 1998).

One program of Ebola immunization, which has been tested in primates, began with a series of DNA injections to initiate cellular immunity (Sullivan and others 2000). The immune response to Ebola proteins was then boosted with an attenuated form of a virus that normally causes colds but has been engineered to express Ebola virus proteins (adenoviral-vector immunization). Adenoviral-vector immunization is effective in inducing protective antibodies as well as cellular immunity. Many vaccinologists think that a vaccine schedule that elicits both types of immune response is likely to give the best results.

Conclusion

Ebola infection was once believed to be limited to isolated outbreaks in distant lands. Forces of globalization, however, potentiate outbreaks anywhere in the world through incidental transmission. Thus, vaccination, which has been one of our most powerful antimicrobial strategies, has assumed greater urgency. Achieving that goal will not be a simple endeavor. Progress in important areas is noted above. However, there may be specific requirements in different regions of the world or for different populations. Nevertheless, the great range of immunological, microbiological, and molecular biological research on vaccine-relevant issues provides hope that the problems can be identified and successfully addressed.

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