

Chapter 3.2

THERAPY OF EBOLA AND MARBURG VIRUS INFECTIONS

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Abstract: The filoviruses, Marburg and Ebola, cause fulminant hemorrhagic fever in humans and nonhuman primates. The agents replicate to high titer in macrophages and dendritic cells, suppressing type I interferon responses, and disseminate to these and other cell types in tissues throughout the body, causing extensive necrosis and inducing an intense systemic inflammatory response resembling septic shock. Effective therapies are urgently needed to deal with laboratory accidents, natural epidemics and the threat of bioterrorism. An immediate goal should be the development of postexposure prophylaxis for persons who have been exposed to Marburg or Ebola virus, but have not yet become ill. This article first reviews current research aimed at blocking individual steps in the filoviral replication pathway, then describes efforts to characterize and modify damaging host responses. No direct inhibitors of filoviral replication have yet been identified, but some interventions that counteract suppression of type I interferon or modify inflammatory responses have shown benefit in laboratory animal models of lethal infection.

1. INTRODUCTION

The filoviruses, Marburg and Ebola, are nonsegmented, negative-strand RNA viruses that cause fulminant hemorrhagic fever in humans and nonhuman primates. In epidemics caused by these agents in central Africa, case fatality rates have ranged from 50 to 90%. Marburg virus was first recognized by the medical community in 1967, when it was introduced into

Europe in a shipment of monkeys from Uganda, infecting 25 vaccine plant workers and 7 of their medical attendants (Martini *et al.*, 1968). Other than a handful of laboratory accidents, all filoviral infections of humans since that time have occurred in Africa. Only a few additional cases of Marburg hemorrhagic fever were detected until 1999, when a large outbreak took place in the Democratic Republic of Congo; a new epidemic in Angola has claimed more than 300 victims at the time of writing.

Ebola virus was first recognized in 1976, when the Zaire and Sudan species caused separate hospital-based epidemics in those two countries (Sanchez, 2001; Bray, 2002). Both agents largely disappeared from view until the mid-1990s, but they have since caused epidemics with increasing frequency (Pourrut *et al.*, 2005). A third Ebola species, Ivory Coast virus, has so far caused only a single known human infection (Formenty *et al.*, 1999). The fourth, the enigmatic Reston agent, caused outbreaks of lethal illness among quarantined macaques imported from the Phillipines from 1989 through 1995 (Jahrling *et al.*, 1990; Miranda *et al.*, 1999). No disease was observed in workers exposed to sick animals, but the agent's virulence for humans remains undetermined.

The animal reservoir of the filoviruses has not been identified. As for the other viruses that cause severe hemorrhagic fever, humans are only accidental hosts (Bray, 2005; Pourrut *et al.*, 2005). The lack of adaptation of these pathogens to humans helps to explain both the rapidly overwhelming nature of infection and the inefficiency of person-to-person transmission, which requires direct contact with virus-containing material (Dowell *et al.*, 1999; Bray and Geisbert, 2005). Nonhuman primates are also highly susceptible to lethal infection, but the rapid progression of illness and high mortality in these animals indicates that they cannot act as natural reservoirs. For unknown reasons, the Zaire species of Ebola virus has been spreading among wild primates in central Africa, and a number of recent outbreaks in humans have been initiated by direct contact with a sick or dead gorilla, chimpanzee or other animal (Peterson *et al.*, 2004; Pourrut *et al.*, 2005; Rouquet *et al.*, 2005).

Even though less than 3000 cases of Marburg and Ebola virus infection have been recognized since their discovery, these agents are a cause of global concern because of the highly lethal nature of infection, the risk that infected individuals will transport these pathogens out of Africa, and the possibility that they could be used as bioterror weapons (Bray, 2003). No specific treatment is currently available for filoviral hemorrhagic fever, so the development of effective therapies is a high priority for biodefense research. Efforts have followed two general approaches (Bray and Paragas, 2002). The first aims to identify pharmacologic agents such as nucleoside analogs, peptides, antisense oligonucleotides or other substances that directly

inhibit viral replication. These efforts have had only limited success, but recent advances in understanding the viral replication pathway may lead to more rapid progress. The other therapeutic strategy attempts to prevent or mitigate illness by modifying host responses. This approach recognizes that the virulence of filoviruses for humans stems in large part from their ability to suppress innate antiviral defenses, particularly the type I IFN response, and their induction of a systemic inflammatory syndrome (Mahanty and Bray, 2004; Bray and Geisbert, 2005). Interventions therefore aim to stimulate innate defenses while blocking damaging inflammatory responses. In contrast to the first approach, this strategy has had some success in protecting filovirus-infected animals, but so far efficacy has been limited to pre- or postexposure prophylaxis.

This chapter begins with a summary of the basic characteristics and replication cycle of the filoviruses, followed by discussions of the clinical features and pathogenesis of filoviral hemorrhagic fever, the nature of host responses to infection, experience with treatment of human disease, and available methods for *in vitro* and *in vivo* drug testing. The remainder of the chapter reviews principal research findings from the two approaches to therapy just described, and concludes by suggesting promising areas for further investigation.

2. FILOVIRAL CLASSIFICATION, VIRION STRUCTURE AND REPLICATION PATHWAY

2.1 Classification

The two filoviral genera, Marburgvirus and Ebolavirus, make up the family *Filoviridae* in the order *Mononegavirales*. The family name is derived from the Latin word *filum* (thread), reflecting the agents' unique filamentous morphology (Figure 1). Four different species of Ebola virus are recognized, as listed above, but all isolates of Marburg virus are currently grouped as one species.

All filoviruses share the same genomic organization, but Marburg and Ebola differ significantly in nucleotide sequence and lack antigenic cross-reactivity (Bray, 2002; Feldmann *et al.*, 2003). Filoviral genomes are approximately 19 kb in length. The linear arrangement of the seven viral genes and the mechanisms of transcription and genome replication resemble those of the better-known rhabdo- and paramyxoviruses. Transcription of each viral gene is controlled by conserved initiation and termination sequences in noncoding regions at the 3' and 5' ends (Sanchez, 2001). The

ends of the genome contain conserved, complementary sequences, which apparently function as cis-acting regulators of genomic replication and transcription and may also play a role in genome packaging.

2.2 Virion structure

Filovirus virions appear as long filamentous threads under the electron microscope (Figure 1). Viral particles vary considerably in length, but have a constant diameter of 80 nm. Because host-cell enzymes cannot transcribe or copy the negative-sense RNA genome, all components of the viral replication complex must be carried within the virion. The central ribonucleoprotein (RNP) core of the virion is comprised of the genomic RNA molecule and its encapsidating nucleoproteins, NP and VP30. Two other proteins, VP35 and the RNA-dependent RNA polymerase, or L protein, are also present within the virion, but in lower copy number. The viral envelope, derived from the host cell membrane, is linked to the RNP by two matrix proteins, VP24 and VP40, which apparently interact with the C-termini of trimeric spikes of the embedded virion surface glycoprotein (GP) (Figure 1).

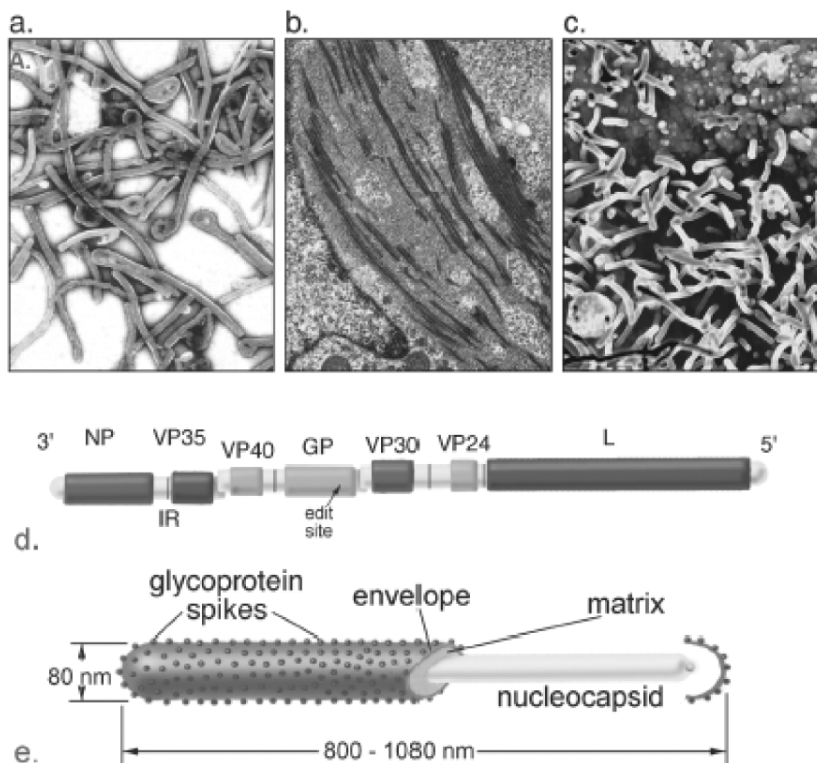


Figure 1: A-C : Ebola Zaire virus. A. Transmission electron micrograph of free virions. B. Cytoplasmic inclusion body within an infected hepatocyte, showing viral nucleocapsids. C. Scanning electron micrograph showing virions budding from the surface of an infected primary human umbilical vein endothelial cell. D. Sequence of genes along the single-stranded negative-sense RNA genome of Ebola virus (see text for abbreviations). Intergenic regions (IR) contain initiation and termination signals. The edit site in the GP gene of Ebola virus consists of a series of 6 adenosine residues. E. Structure of an Ebola or Marburg virion. The RNP core consists of the genomic RNA molecule and its encapsidating nucleoproteins, NP and VP30, which are linked by matrix proteins, VP24 and VP40, to the virion envelope, a lipid bilayer derived from the host cell. The virion surface contains embedded trimeric spikes of the viral GP. (Images A-C courtesy of Tom Geisbert, USAMRIID.).

2.3 Replication Cycle

Infection begins with the attachment of the virion to the cell surface (Fig. 2). A specific receptor molecule has not been identified. Studies employing pseudotyped retroviruses bearing Marburg or Ebola GP on their surface indicated that the human folate receptor- α was a filovirus binding site, but subsequent investigations failed to support this hypothesis (Chan *et al.*, 2001; Simmons *et al.*, 2003).

The asialoglycoprotein was also identified as a receptor for Marburg virus, but because this molecule is not expressed by a number of cell lines that are susceptible to filovirus infection, its role in pathogenesis remains uncertain (Becker, Spiess, and Klenk, 1995). In fact, it now appears that the initiation of filoviral infection may not require a specific receptor, since the heavily glycosylated filoviral GP can bind to cell-surface lectins, including the dendritic cell surface protein DC-SIGN and macrophage C-type lectin (Marzi *et al.*, 2004; Takada *et al.*, 2004).

Following attachment, filovirus virions exploit the cellular endocytosis machinery, in which engulfment in an endosome is followed by a drop in pH, a conformational change in GP, fusion of viral and cell membranes and release of the genome and internal virion proteins into the cytoplasm. Transcription is then performed by a replication complex, which in the case of Ebola virus is made up of four proteins (VP35, VP30, NP and L); VP30 is not required for Marburg virus replication (Boehmann *et al.*, 2005; Theriault *et al.*, 2004). Transcription initiates at the 3' end of the genome, resulting in synthesis of a leader RNA and seven polyadenylated mRNAs (Sanchez, 2001). Accumulation of the first two gene products (NP and VP35) in some way triggers a switch to the production of full-length, positive-sense "antigenomes", which are then used as templates for genome synthesis. The production of new genomes and their encapsidation leads to the accumulation of nucleocapsids in large cytoplasmic inclusion bodies visible in stained tissue sections (Figures 1 and 2).

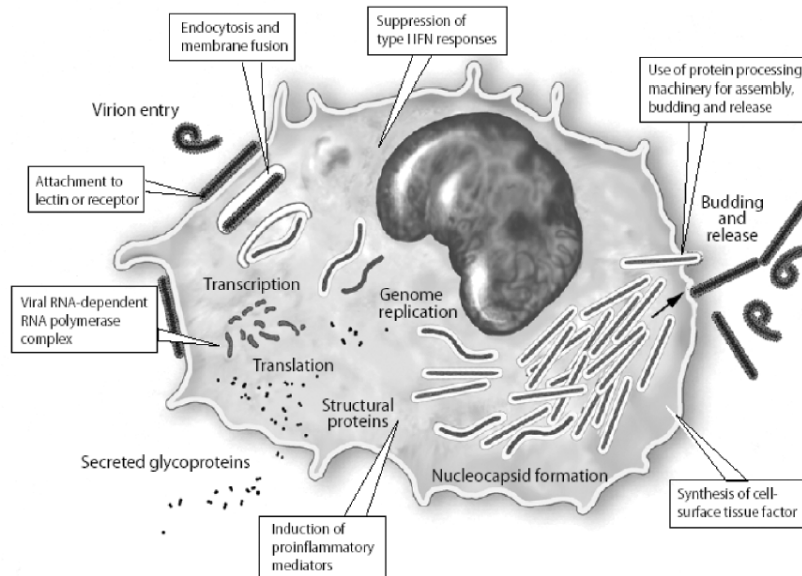


Figure 2: The filovirus replication cycle. Boxed labels denote processes that may represent targets for therapeutic intervention. Virions attach to cell-surface lectins and/or a specific receptor, and are then taken up by endocytosis. Fusion with the cell membrane within endosomes releases the RNP and other components of the replication complex into the cytoplasm. Viral mRNA is translated by the host cell machinery to produce virion structural proteins, plus, in the case of Ebola virus, a C-terminally truncated, secreted form of the virion surface glycoprotein (GP). Full-length GP is glycosylated in the Golgi apparatus, transported to the cell membrane and cleaved to form two units that remain linked by a disulfide bond as GP1,2. Virion assembly begins with the encapsidation of new genomic RNA molecules by NP and VP30. Production of filamentous virions takes place at the cell membrane, in association with lipid rafts. The process is apparently driven by VP40, which interacts with cellular proteins, bringing about a conformational change that results in its self-association into a progressively enlarging structure that forms the internal scaffolding of the virion envelope. VP24 may help link this structure to the central RNP core and/or to the C-terminal cytoplasmic tails of GP1,2.

The assembly of new virions begins on the inner surface of the plasma membrane, where nucleocapsids apparently become linked through two matrix proteins, VP24 and VP40, to the cytoplasmic tail of GP2 molecules embedded in the cell surface. This process localizes to cholesterol- and glycosphingolipid-rich areas termed lipid rafts, which may provide a stabilizing structure for the assembly process (Bavari *et al.*, 2002; Aman *et al.*, 2003; Panchal *et al.*, 2003). Like a number of other pathogens, Ebola and Marburg virus apparently bring about their own release from the cell by “hijacking” systems normally employed for degradation or excretion of proteins. Budding of new virions appears to be driven primarily by VP40,

which is able to self-oligomerize on contact with lipid bilayers. The expression of that gene alone in transfected cells results in the extrusion of filamentous virus-like particles (VLP) that closely resemble infectious virus; their production is markedly enhanced by co-expression of GP in the same cells (Jasenosky and Kawaoka, 2004; Licata *et al.*, 2004; Irie, Licata, and Harty, 2005). Two overlapping “late-domain” motifs, PTAP and PPEY, in the N terminus of VP40 interact with proteins involved in the endosomal pathway: the ubiquitin ligase Nedd4, which marks proteins for degradation or excretion, and Tsg101, a component of the ESCRT-1 complex that directs ubiquitinated proteins to endosomes (Jasenosky and Kawaoka, 2004). Optimum VLP formation requires the presence of these sequences, suggesting that specific interactions between virus and host proteins are required for virion release and may represent targets for antiviral therapy (Irie, Licata, and Harty, 2005). The primary product encoded by the Ebola virus GP gene is a truncated form of the protein that is released from infected cells (“soluble” glycoprotein, sGP) (Feldmann *et al.*, 1999). Synthesis of the full-length virion surface GP requires the insertion of an additional adenosine at an “editing site” during transcription (Volchkov *et al.*, 1995). The release of large amounts of sGP has been proposed to play a role in the remarkable virulence of Ebola virus for primates, but since Marburg virus is just as virulent, but does not encode a similar protein, the actual contribution of sGP to pathogenesis remains unknown. The GP of both Ebola and Marburg virus becomes abundantly glycosylated with N- and O-linked carbohydrates during transit through the Golgi apparatus, and is then cleaved by a cellular furin-like enzyme to produce two units, extracellular GP1 and transmembrane GP2, that remain linked by a disulfide bond as GP1,2 (Volchkov *et al.*, 1998).

3. FILOVIRAL HEMORRHAGIC FEVER

3.1 Clinical Syndrome

Marburg and Ebola viruses cause similar diseases in humans (Martini, 1973; Bwaka *et al.*, 1999; Mahanty and Bray, 2004). After an incubation period of some 5-10 days, illness begins abruptly, with headache, fever, muscle pain, vomiting, diarrhea and other nonspecific signs and symptoms. Over the next week, persistent fever and worsening prostration and stupor are accompanied by a fall in blood pressure. Hemorrhagic phenomena generally take the form of petechiae, ecchymoses, conjunctival hemorrhages and oozing from venipuncture sites. Massive bleeding is rare. The onset of

clinical deterioration is marked by tachypnea, anuria, a fall in body temperature and the development of coma as progressive hypotension leads to intractable shock. Death usually occurs 6-9 days after the onset of symptoms.

Data from African patients infected with Ebola Zaire virus indicate that fatal infection is characterized by a steadily rising titer of circulating virus and the absence of a detectable antibody response, while in survivors, peak viral titers are significantly lower and IgM anti-Ebola antibodies are detectable during the second week of illness (Ksiazek *et al.*, 1999; Sanchez *et al.*, 2004; Towner *et al.*, 2004). Limited data from Africa indicate that persons who survive infection also show evidence of early, strong proinflammatory cytokine responses, while in fatal cases these are seen later in infection, often accompanied by anti-inflammatory mediators such as interleukin(IL)-10 (Baize *et al.*, 1999, 2002). Studies performed during outbreaks in Gabon also revealed the presence of proinflammatory cytokines and Ebola-specific IgG antibodies in blood samples from some persons who were in direct contact with Ebola Zaire patients, but did not become ill, suggesting that these individuals were able to eliminate infection through an early, brisk inflammatory response (Leroy *et al.*, 2000, 2001).

Because of the extreme difficulty of performing clinical studies under outbreak conditions in central Africa, most data on the clinicopathologic features of filoviral hemorrhagic fever come from studies in laboratory animals, especially from cynomolgus macaques infected with Ebola Zaire virus from the 1995 outbreak (Bray *et al.*, 2001; Geisbert *et al.*, 2003b, c). Inoculation of a standard 1000 pfu challenge is followed in 3-4 days by the onset of fever and diminished activity, the appearance of a hemorrhagic macular rash on day 4-5, obtundation by day 6 and death on day 7-8. As in humans, mild hemorrhagic phenomena are common, but profuse bleeding is rare. Virus is detectable in the serum by day 3, and titers exceed 10^7 pfu/mL by day 5. Infection is also characterized by lymphopenia, neutrophilia, DIC and massive "bystander" apoptosis of lymphocytes and NK cells. The model thus appears to differ from Ebola hemorrhagic fever in humans principally in that death occurs in all cases, usually 3-4 days after the onset of visible symptoms, while humans are ill for 1-2 weeks and a small percentage survive infection.

Pathologic changes in the macaque model include the infection and necrosis of macrophages and dendritic cells in the spleen, liver, lymph nodes and other lymphoid tissues; multifocal necrosis of the liver, adrenal cortices and other organs; and extensive apoptosis of lymphocytes. Abnormalities in clinical laboratory tests include neutrophilia, lymphopenia, marked thrombocytopenia and prolongation of coagulation times, with circulating fibrin degradation products and other features of disseminated intravascular

coagulation (DIC). Serum levels of liver-associated enzymes, particularly aspartate aminotransferase, are elevated.

3.2 Target cells and viral dissemination

Like all microbial pathogens, filoviruses are confronted by macrophages and dendritic cells as soon as they enter the body. Both types of cells employ a variety of innate immune mechanisms for antiviral defense, but dendritic cells also specialize in initiating adaptive immune responses. Far from being destroyed by these cells, filoviruses replicate within them and impair their function, so that they are able to initiate inflammation and coagulation, but cannot prevent viral dissemination (Figure 3) (Mahanty and Bray, 2004; Bray and Geisbert, 2005). Viral replication and spread are aided by suppression of type I interferon (IFN) responses (see below). In consequence, infection spreads rapidly to similar cells in tissues throughout the body, causing the release of massive quantities of proinflammatory cytokines, chemokines and other mediators that produce a syndrome of refractory hypotension and DIC resembling septic shock (Hensley *et al.*, 2002; Bray and Mahanty, 2003). Infected dendritic cells are unable to undergo maturation or initiate adaptive immune responses (Mahanty *et al.*, 2003b; Bosio *et al.*, 2004).

In addition to these indirect effects, filoviruses cause direct tissue injury, since replication in macrophages, dendritic cells and parenchymal cells of the liver, adrenal cortices and other organs results in extensive necrosis, contributing to fatal disease (Figure 3). Careful studies in nonhuman primates have shown that endothelial cells remain uninfected by virus until the later stages of disease, perhaps only becoming susceptible to infection after they have become activated by circulating inflammatory mediators (Geisbert *et al.*, 2003c). Although lymphocytes also remain uninfected, they undergo massive apoptosis through a “bystander” mechanism, further impairing immune function (Geisbert *et al.*, 2000; Geisbert *et al.*, 2003b; Reed *et al.*, 2004).

3.3 Host response to infection

The principal early host defense against viral infection is the production and release of type I IFN, which binds to cell-surface receptors and sets off a train of reactions, including the expression of a large number of new proteins, that result in an “antiviral state” (Katze *et al.*, 2002; Weber *et al.*, 2004). Not unexpectedly, viruses have evolved a variety of mechanisms to

block or evade these responses. Considerable evidence now indicates that suppression of IFN responses plays a major role in the ability of the filoviruses to cause rapidly overwhelming infection in primates.

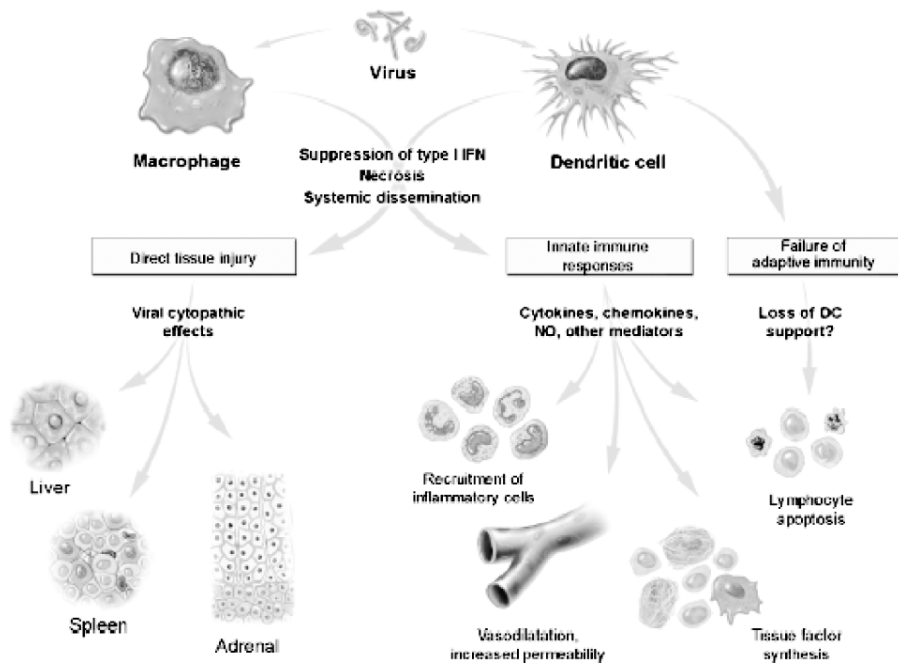


Figure 3: Pathogenic mechanisms of filoviral hemorrhagic fever, based on studies of lethal Ebola Zaire virus infection of macaques. Virus initially replicates in macrophages and dendritic cells (DC), causing their necrosis; suppression of type I IFN production aids viral dissemination. Proinflammatory cytokines, chemokines, tissue factor and other mediators produced by these cells cause increased vascular permeability and coagulopathy. Filoviruses also spread to parenchymal cells in the liver, adrenal cortex and other organs, causing multifocal necrosis, while destruction of macrophages and DC causes extensive injury to the spleen and other lymphoid tissues. Massive ‘bystander’ apoptosis of lymphocytes is apparently brought about through mediator effects and loss of DC support. NO: nitric oxide.

Most data on viral interactions with the type I IFN system come from studies in cultured cells. Primary human macrophages infected with Ebola Zaire virus produced only small amounts of IFN- α late in infection, and failed to release IFN when exposed to double-stranded RNA (dsRNA)

(Harcourt *et al.*, 1998, 1999). Basler *et al.* have identified such suppression with the Ebola VP35 protein, which can functionally replace the influenza NS1 to inhibit IFN production by infected cells (Basler *et al.*, 2000). VP35 appears to act by blocking the recognition of dsRNA, preventing the phosphorylation and nuclear translocation of interferon response factor-3 and the transcription of a series of IFN-related genes (Basler *et al.*, 2003; Hartman, Towner, and Nichol, 2004; Basler, 2005). Further confirmation of the anti-IFN activity of VP35 was provided by a study in which a recombinant alphavirus encoding the protein induced significantly less IFN- α production by infected cells than the control virus (Bosio *et al.*, 2003).

The mouse model of Ebola Zaire virus infection has been particularly useful in exploring interactions between filoviruses and type I IFN responses (Bray, 2001; Bray *et al.*, 2002; Gupta *et al.*, 2004). In marked contrast to primates, normal adult mice are solidly resistant to all wild-type filoviruses. However, they can be rendered sensitive to lethal infection by Ebola Zaire and Ebola Sudan virus through treatment with anti-IFN- α antibodies, and knockout mice lacking the STAT-1 protein or the cell-surface IFN- α/β receptor are rapidly killed by these and other filoviruses (Bray, 2001). Further evidence of the critical role of type I IFN responses has been obtained using a variant of Ebola Zaire virus that is lethal for adult mice, which was obtained through sequential passage in suckling mice of virus from the 1976 outbreak (Bray *et al.*, 1998; Gibb *et al.*, 2001). The LD₅₀ of this “mouse-adapted virus” by the intraperitoneal route is approximately one virion; infection induces only a low level of IFN- α production, that first becomes detectable in serum when the animals are already ill. Remarkably, mice remain healthy when inoculated subcutaneously with the same virus; in this case, infection results in the rapid production of large amounts of IFN- α (Mahanty *et al.*, 2003a).

3.4 Experience with human therapy

Almost all patients with Marburg or Ebola hemorrhagic fever in African outbreaks have received general supportive treatment, and only a few descriptions of attempts to provide specific therapy can be found in the medical literature. One researcher accidentally exposed to Ebola Sudan virus in the laboratory became severely ill, but survived infection after treatment with human convalescent serum and IFN- α (Emond *et al.*, 1977). It is not clear that either therapy contributed to the fortunate outcome. A Russian laboratory worker accidentally infected with Marburg virus also survived after prolonged treatment (Nikiforov *et al.*, 1994). Because the nature of his infection was not recognized until after the onset of illness, he did not

receive immune serum or IFN, but underwent several rounds of extracorporeal blood treatment with hemosorbents and dialysis. These measures produced transient improvements in his condition, but it is again difficult to conclude from this single case that therapy was responsible for survival. Several Russian laboratory workers who were possibly exposed to Ebola Zaire virus in the laboratory have been treated with a preparation of equine anti-Ebola immunoglobulin and recombinant IFN (see below) (Kudoyarova-Zubavichene *et al.*, 1999).

The only published report of an attempt to provide specific therapy during the course of a natural outbreak describes the use of whole blood transfusions from convalescent survivors toward the end of the 1995 Ebola Zaire epidemic (Mupapa *et al.*, 1999). The initial impression was highly favorable, since seven of the eight laboratory-confirmed patients who received blood survived infection. However, subsequent analysis showed that all of these patients would have been expected to survive, even without therapy, since they had already lived for at least 11 days since the outset of symptoms at the time they were treated (Sadek *et al.*, 1999).

4. EVALUATING NEW DRUGS FOR FILOVIRAL INFECTIONS

4.1 *In vitro* testing

The evaluation of a candidate antiviral drug for filoviral infections is commonly performed by assessing its ability to prevent viral cytopathic effect, as measured by the uptake of neutral red dye or another indicator molecule by virus-infected cells in culture. Such work must necessarily be performed under BSL-4 containment. Recently, this approach has been supplemented by the construction of a recombinant Ebola Zaire virus encoding green fluorescent protein (GFP), that can potentially be used to obtain a direct read-out of the extent of viral replication (Towner *et al.*, 2005).

Because few laboratories possess the level of biocontainment required to work with filoviruses, other methods have been developed to study portions of the filoviral replication pathway. One approach is the construction of “pseudotyped” viruses that encode the replicative machinery of vesicular stomatitis virus (VSV), murine leukemia virus or human immunodeficiency virus (HIV)-1, but express the Marburg or Ebola GP on the virion surface (Takada *et al.*, 1997; Kobinger *et al.*, 2001; Manicassamy *et al.*, 2005;

Yonezawa *et al.*, 2005). These chimeric agents provide valuable information about such GP-related functions as virion binding, fusion and entry into target cells, and can therefore be used for the initial evaluation of inhibitors of these activities.

A method that can be used to study transcription and genome replication is the “minigenome” system, in which cultured cells are transfected with plasmids encoding individual components of the filoviral replication complex. A substrate for transcription is provided by generating an RNA transcript containing a reporter gene flanked by filoviral initiation and termination sequences (Muhlberger *et al.*, 1999; Weik *et al.*, 2002; Groseth *et al.*, 2005). Although somewhat cumbersome, this system could potentially be used without the constraints of BSL-4 containment to identify compounds that inhibit the viral RNA-dependent RNA polymerase, induce a high error rate or cause chain termination.

Transfection of cultured cells with plasmids encoding filoviral proteins can also be employed to study later steps in replication, including the assembly and budding of new virions. As described above, this method has been used to demonstrate that expression of Ebola virus VP40 is sufficient to cause production and release of filamentous virus-like particles from the cell surface, and that specific late-domain sequences in the N-terminus of VP40 are essential for this process (Jasenosky and Kawaoka, 2004). This system could be used to screen for inhibitors of protein-protein interactions involved in the budding process.

Another approach to finding effective therapies for filovirus infections is based on the study of less virulent viruses with similar replication mechanisms. This strategy recognizes that it would be more financially advantageous for a pharmaceutical company to develop a drug that is active against both filoviruses and another, more common pathogen than to identify a compound that only inhibits Ebola and Marburg virus. One such “surrogate” agent is respiratory syncytial virus (RSV), a nonsegmented, negative-strand RNA virus that causes severe illness in infants and immunocompromised adults (Huggins *et al.*, 1999). It should be noted, however, that although the use of surrogates may identify lead compounds for further study, such predictions are not always accurate. For example, even though ribavirin is active against RSV, it does not inhibit filovirus replication.

4.2 Animal models

Three types of laboratory animals – mice, guinea pigs and nonhuman primates – are in use for testing antiviral drugs and vaccines against Marburg

and Ebola infection and to study filovirus pathogenesis. Although the principal target cells of infection are similar in these diverse species, these animals differ significantly in their inherent resistance to filoviruses. Nonhuman primates are exquisitely sensitive to all wild-type Marburg and Ebola isolates, including Ebola Reston virus, but guinea pigs and mice can only be used as experimental models after a virus has been “adapted” to them through serial passage. This difference in innate resistance to infection apparently reflects the ability of filoviruses to suppress type I IFN responses in primates, but not in rodents; it frequently causes divergent outcomes in tests of drug and vaccine efficacy (Geisbert *et al.*, 2002).

Filovirus replication in mice can be studied by a number of approaches. Marburg virus and the Zaire and Sudan species of Ebola virus cause lethal infection in newborn mice, but do not cause visible illness in adult animals. Severe combined immunodeficient (SCID) mice also differ from primates in their sensitivity to filoviruses, in that they develop slowly progressive disease after inoculation of most filoviral species, dying 3-6 weeks after challenge. By contrast, gene-knockout mice lacking the STAT-1 protein or the cell-surface IFN- α/β receptor develop rapidly lethal disease after injection of the same viruses (Bray, 2001). Most recent studies in mice have employed the mouse-adapted variant of Ebola Zaire '76 virus described above, that causes rapidly lethal disease in normal adult mice when inoculated by the intraperitoneal route (Bray *et al.*, 1998). The pathologic features of infection resemble those in primates, except that signs of coagulopathy are much less prominent (Bray *et al.*, 2001; Gibb *et al.*, 2001). As noted, even though normal mice can be killed by the intraperitoneal injection of a single particle of this “mouse-adapted virus”, they are solidly resistant to massive doses of the same virus injected subcutaneously – a property that makes this model especially useful for studying mechanisms of susceptibility and resistance to filovirus infection.

Guinea pigs are inherently less resistant to filoviral infections than mice, since they develop a mild febrile illness after inoculation with wild-type Marburg or Ebola Zaire or Sudan virus, and these agents can readily be adapted to lethal virulence in guinea pigs through serial passage. The major pathologic features of fatal infection resemble those in mice and primates, except that signs of coagulopathy are again lacking (Connolly *et al.*, 1999). Guinea pigs have frequently been employed for vaccine testing, but the lack of specific reagents or detailed knowledge of immune function makes them less useful for this purpose than mice. Their comparatively large size is also a distinct disadvantage for antiviral drug testing, since even a young animal may require 10-20 times as much of an experimental compound as a weanling mouse.

In marked contrast to rodents, nonhuman primates are highly susceptible to severe or lethal hemorrhagic fever after challenge with wild-type filoviruses, including Ebola Reston. Cynomolgus and rhesus macaques are used most often for laboratory studies. Ebola Zaire virus infection is uniformly lethal for these animals, while the Sudan and Reston species and Marburg virus cause a similar disease, but with a longer clinical course and generally less than 100% mortality. Interestingly, the mouse-adapted variant of Ebola Zaire virus appears to be somewhat attenuated for primates, since two of three macaques inoculated with the agent developed only low-level viremia and survived infection (Bray *et al.*, 2001).

5. APPROACHES TO THERAPY OF FILOVIRAL INFECTIONS

Effective therapies are needed to deal with two situations. The more urgent, but perhaps more easily achievable goal is to develop forms of postexposure prophylaxis that can be used to treat persons who have been infected with Marburg or Ebola virus, but have not yet become ill. Such treatment could be given to individuals who have been in direct contact with patients in an African outbreak or to a researcher accidentally infected in the laboratory, and might also be used to protect people exposed to a filovirus in a bioterror attack. Postexposure prophylaxis could potentially be achieved in two different ways: treatment with a compound that directly inhibits filovirus replication, or administration of a substance that bolsters innate immunity and blocks damaging host responses. Possible targets for both approaches are discussed below.

The second, far more difficult problem is to develop specific interventions that can rescue persons who are already ill with filoviral hemorrhagic fever. In contrast to treatment during the incubation period, halting the progression of disseminated infection probably cannot be achieved solely by modifying host responses, but will require therapy that blocks viral replication. Given the extreme severity of these conditions, a combination of pharmacologic agents with different mechanisms of action may be required to cure patients with full-blown disease.

5.1 Direct inhibition of viral replication

The following section proceeds through the stages in the filoviral replication cycle, from attachment to the cell surface through the release of new progeny virions, and describes current efforts to develop inhibitors of

each step. The discussion includes both “small molecules,” such as nucleoside analogues, that are produced through chemical synthesis, and substances generated in biological systems, such as antibodies and lectins.

5.1.1 Prevention of virion binding

The fact that high viremia in the absence of an antibody response is predictive of death in patients with Ebola hemorrhagic fever suggests that neutralization of circulating virus would improve survival. To date, efforts to develop such therapies have been based principally on the use of polyvalent or monoclonal antibodies. The most experience with polyvalent antiserum has been obtained with immunoglobulin produced in Russia by hyperimmunizing horses, sheep or goats with Ebola Zaire virus (Kudoyarova-Zubavichene *et al.*, 1999). These studies suggest that the efficacy of antibody therapy depends both on the donor species and the animal model in which it is tested. Thus, pre- or immediate postexposure treatment with equine anti-Ebola immunoglobulin prevented the death of most baboons infected with Ebola Zaire virus and protected guinea pigs infected with an adapted variant of the same agent, but even large doses of the same product administered soon after challenge did no more than delay the onset of illness in mice and cynomolgus macaques by 1-2 days (Jahrling *et al.*, 1996, 1999). Despite these mixed reviews, this immunoglobulin is now approved in Russia for the treatment of laboratory-acquired Ebola virus infections. It has been administered to several workers thought to have been exposed to Ebola virus, who remained well, but without proof that they were actually infected (Kudoyarova-Zubavichene *et al.*, 1999).

These and other results suggest that antibody therapy is more likely to be successful in species in which infection is characterized by a longer incubation period and lower peak serum viral titer (guinea pigs and baboons) than in animals in which the same virus causes rapidly progressive disease with a high peak viral load (mice and monkeys). The efficacy of heterologous antiserum may also be limited by immune recognition and clearance of the foreign protein and by incompatibility between the donor IgG and the recipient's Fc receptors (Wilson *et al.*, 2001). The latter consideration suggests that efficacy can be improved by using homologous immune serum, and in contrast to the results cited above, passive transfer of serum from immune to naïve mice protected the recipients against mouse-adapted Ebola Zaire virus (Gupta *et al.*, 2001). Treatment up to 2 days after infection was remarkably effective, preventing the death of both normal and SCID mice.

These experimental successes with polyvalent antisera have led to efforts to develop monoclonal antibodies (mabs) for use in humans. Initial

experiments in mice showed that mabs specific for any of five different epitopes on the Ebola Zaire GP were protective when inoculated up to 2 days after virus challenge (Wilson *et al.*, 2000). Some antibodies were protective even though they failed to neutralize virus in plaque-reduction assays, suggesting that the *in vitro* test does not always reflect mechanisms of protection *in vivo*. Human mabs that react strongly with the Ebola Zaire GP, sGP or NP have been produced from phage-display libraries constructed using mRNA from survivors of the 1995 Ebola Zaire outbreak (Maruyama *et al.*, 1999a, b). One antibody that neutralized Ebola Zaire virus *in vitro* at an inhibitory concentration of 0.3 µg/ml also protected guinea pigs when given before or within an hour after virus challenge (Parren *et al.*, 2002). Some treated animals survived despite developing a high viremia, providing further evidence that antibodies can act through mechanisms other than direct neutralization. However, successful protection of nonhuman primates with this or other antibodies has yet to be reported.

Another approach to preventing the binding of a filovirus to target cells involves the synthesis of bispecific IgG, in which one arm of the antibody molecule binds a viral antigen, while the other has specificity for a host protein. One such “heteropolymer” has been described that recognizes both the Marburg virus GP and the complement receptor-1 on human erythrocytes (Nardin *et al.*, 1998). Ideally, such a molecule could clear virions from plasma by attaching them to the surface of red blood cells, which would then be taken up and eliminated by phagocytes in the spleen and other tissues. *In vitro* tests showed that such antibodies could bind inactivated Marburg virus to erythrocytes, but their *in vivo* efficacy has not been reported.

Since filoviruses may make use of DC-SIGN, macrophage surface C-type lectin and other lectins to attach to target cells, another therapeutic strategy involves administering naturally occurring lectins that bind carbohydrate moieties on the filoviral surface GP and can thus serve as “decoys” for the virus, in a manner similar to naturally occurring mannose-binding lectin, which serves as a natural antimicrobial defense in the bloodstream (Ji *et al.*, 2005). Proof-of-concept studies have employed cyanovirin-N, an 11K protein produced by cyanobacteria, which has attracted interest because it binds high-mannose oligosaccharides on the human immunodeficiency virus (HIV) gp120 with high affinity and neutralizes some isolates of influenza virus (Boyd *et al.*, 1997; O’Keefe *et al.*, 2003). Even though cyanovirin has only limited activity against Ebola Zaire virus in cell culture, it was still able to slow the progression of disease in mice (Barrientos *et al.*, 2003; Barrientos and Gronenborn, 2005). Efforts are now under way to characterize the oligosaccharides on the Marburg and Ebola GP that are recognized by CV-N and to isolate other naturally occurring lectins with higher affinity for filoviral GPs.

Another approach to blocking infection of target cells by Marburg or Ebola virus is to administer a substance that can compete for binding to DC-SIGN or other C-type lectins. This has recently been attempted using dendrimer technology, in which a hyperbranched polymer molecule is conjugated to multiple units of a high-mannose oligosaccharide (Lasala *et al.*, 2003; Rojo and Delgado, 2004). In proof-of-concept experiments using pseudotyped retroviral particles expressing the Ebola virus GP, a dendrimer molecule blocked virus entry at an IC_{50} of less than 1 μ M. Evaluation against live virus or in an animal model has not yet been reported.

5.1.2 Prevention of membrane fusion and entry

Once a filovirus virion has bound to a target cell, its surface GP must undergo a conformational change to permit the viral envelope to fuse with the cell membrane, releasing the RNP and viral proteins into the cytoplasm. Some pathogens, such as human immunodeficiency virus (HIV)-1, carry out fusion at the cell surface, but filoviruses apparently perform this process within endosomes, in a step requiring acidification (Wool-Lewis and Bates, 1998; Chandran *et al.*, 2005).

Efforts to develop filoviral fusion inhibitors have been based in part on the successful “T20” HIV-1 inhibitor (Cooley and Lewin, 2003). In situ mutagenesis of the Ebola Zaire GP expressed by pseudotyped viruses has identified regions of the protein that interact with each other to bring about a conformational change and membrane fusion, and are required for viral entry (Watanabe *et al.*, 2000). An oligopeptide mimicking one of these fusion sequences blocked infection by a pseudotyped retrovirus, but the 50% inhibitory concentration exceeded 1 mg/ml, a level that probably could not be achieved *in vivo*. Successful therapy with a fusion inhibitor may therefore require the development of methods of directing such peptides into endosomes, so as to increase their concentration at sites of fusion.

As noted, filovirus entry into target cells has also recently been shown to involve cleavage of GP1 by cellular cathepsins (Chandran *et al.*, 2005). It is not yet known if this process is essential for infection, or whether alternative entry pathways may be available to these pathogens. Attempts to block infection *in vivo* through treatment with cathepsin inhibitors, or the characterization of infection in knockout mice lacking these enzymes, has not yet been reported.

5.1.3 Interference with transcription and genome replication

No compounds have yet been identified that directly inhibit the filoviral RNA-polymerase complex. Although the nucleoside analog ribavirin blocks transcription of a wide range of DNA and RNA viruses, including a number of nonsegmented negative-sense RNA agents, it does not inhibit filoviruses. Thus, treatment with ribavirin failed to alter the course of illness of Ebola- or Marburg-infected guinea pigs or monkeys infected with Ebola Zaire virus (Huggins *et al.*, 1999; Ignatiev *et al.*, 2000). The reason for its lack of activity against filoviruses is not clear.

Another approach that has been successfully employed to inhibit transcription and genome replication of some negative-strand RNA viruses makes use of antisense oligonucleotides complementary to sequences in viral genomic or mRNA. In the case of RSV, for example, inhibition was achieved by covalently linking the antisense molecule to a 2'-5' oligoadenylate sequence, so that binding activated cellular 2'-5' oligoadenylate-dependent RNase L, resulting in cleavage of the RNA target (Torrence *et al.*, 1997; Adah *et al.*, 2001). However, the same approach failed to demonstrate sequence-specific inhibition of Ebola Zaire virus replication (M Bray, P Torrence, unpublished data). Effective antisense therapy may require improved identification of critical regions of the viral genome that are accessible to oligonucleotide binding, stabilization of antisense molecules and development of methods to deliver them efficiently into infected cells.

5.1.4 Prevention of viral mRNA cap methylation

Production of mRNA for efficient translation in eukaryotes requires the attachment of a 5'-5'-linked guanosine cap to the 5' end of the growing mRNA strand, followed by methylation of the cap and adjacent nucleotides at one or more sites by (guanine-7-)methyltransferase. For the latter process, S-adenosylmethionine (SAM) is the methyl donor, and the reaction is driven forward by removal of the product, S-adenosylhomocysteine (SAH), through its hydrolysis by SAH hydrolase. Since the mRNA of many viruses is also capped, inhibitors of the latter enzyme can therefore indirectly block viral replication by causing the cytoplasmic SAH/SAM ratio to rise, impairing methylation (Cools and De Clercq, 1990; Oxenrider *et al.*, 1993).

A number of adenosine analogs have been identified that act through this mechanism to suppress the replication of a broad range of DNA and RNA viruses, including the filoviruses (De Clercq, 1987, 1998; Huggins *et al.*,

1999). Two of them, carbocyclic 3-deaza-adenosine (C-c3Ado) and 3-deazaneplanocin A (c3-Npc A), have *in vitro* 50% inhibitory concentrations (IC₅₀) against Ebola Zaire virus of 30 and 2 μ M, respectively. Both were highly effective in treating Ebola-infected mice when administered thrice daily, beginning on day -1, 0 or +1, but treatment begun on day 2 was less protective (Huggins *et al.*, 1999). Unexpectedly, equivalent or better results were obtained by treating mice once, with a somewhat larger, but nontoxic dose of either drug, on any day from 0 to +2 (Bray *et al.*, 2000). A single dose given as early as 2 days before virus challenge was partially protective.

At present, c3-Npc A is the most potent form of postexposure prophylaxis for Ebola Zaire infection in mice, since a single dose of 1 mg/kg prevents illness and death. However, it is clear that the mechanism of action involves more than just impaired cap methylation, since the beneficial effect can be eliminated by administering antibodies to murine IFN- α/β . As discussed below, further experiments showed that treatment causes massively increased production of IFN- α in infected, but not in uninfected animals (Bray *et al.*, 2002). Unfortunately, trials in nonhuman primates infected with Ebola Zaire virus did not result in either enhanced IFN production or mitigation of illness; the cause of this species-specific difference in drug effect is not known.

5.1.5 Interference with protein processing, viral assembly and exit

Pharmacologic blockade of a viral protease has proven to be a successful strategy for HIV therapy, but it does not appear to be a viable option for the filoviruses, since they do not encode a protease. On the other hand, host-cell enzymes that process viral proteins may constitute a therapeutic target. As noted above, cleavage of GP by host-cell cathepsins occurs during viral entry, and another proteolysis event occurs during maturation of the nascent protein, when GP is cleaved into GP_{1,2} by a cellular furin-like protease (Volchkov *et al.*, 1998). However, even if these steps are essential for filoviral maturation, it appears doubtful that effective treatment could be based entirely on inhibition of these ubiquitous cellular enzymes.

Other types of interactions between viral and host proteins may be more rewarding targets for antiviral attack. Current interest focuses on VP40, which appears to be the “driver” behind virion assembly. As described above, the expression of VP40 in plasmid-transfected cells is sufficient to cause the extrusion of filamentous VLPs from the cell surface (Harty *et al.*, 2000; Jasenosky *et al.*, 2001; Jasenosky and Kawaoka, 2004). Since VP40 interacts with specific components of the cellular protein-processing machinery, including Nedd4 and Tsg101, it is possible that a compound that

interferes with these protein-protein interactions could block viral replication. Harty *et al.* used this approach to show that budding of vesicular stomatitis virus (VSV) is blocked by treatment with proteasome inhibitors (Harty *et al.*, 2001), but this strategy has not yet been tested for the filoviruses.

5.1.6 Destruction of virus infected cells

The expression of viral antigens on the surface of infected cells makes them vulnerable to destruction by the immune system. Antibodies to viral antigens can contribute to this process by enhancing complement activation and facilitating killing by natural killer cells. Although the virion GP is assumed to be the most important target for such antibodies, other virion components may also become accessible to immune recognition in the course of virion assembly and budding. Limited evidence of this was provided by the observation that mabs specific for the Marburg virus VP40 protein caused complement-mediated lysis of infected cells, even though they did not neutralize the virus *in vitro* (Razumov *et al.*, 1998). In a single reported experiment, these antibodies protected guinea pigs against lethal Marburg virus challenge.

Two other strategies that could be used to attack filovirus-infected cells also involve the use of antibodies that recognize antigens on the cell surface. The first is based on studies in the HIV field, which have shown that a variety of toxins coupled to antibodies against viral antigens (“immunotoxins”) are capable of destroying virus-infected cells (Berger *et al.*, 1998; Pincus *et al.*, 2001). Recently this approach has been extended to include the labelling of monoclonal antibodies with an alpha-particle-emitting radionuclide, which has shown efficacy in eliminating infected cells in murine models of bacterial and fungal infection (Dadachova *et al.*, 2004).

The other approach to attacking infected cells takes advantage of the fact that the assembly and budding of new virions may result in the display of cellular molecules that are normally concealed from the immune system. One of these, phosphatidylserine, is the target of a monoclonal antibody that has shown promise in experimental cancer therapy (Ran *et al.*, 2005), and is now being tested for antiviral activity. One caveat regarding these approaches to filovirus therapy is that their potential ability to cause the simultaneous destruction of a vast number of infected host cells may result in severe tissue damage, particularly hepatotoxicity.

5.2 Modulation of host response

Detailed studies in mice and nonhuman primates are helping to define the mechanisms by which filoviruses cause rapidly lethal disease. Mouse experiments have shown that suppression of type I IFN responses plays a critical role in permitting viral dissemination. Studies in nonhuman primates, by contrast, have probably been most useful in revealing that inflammatory mediators released by virus-infected macrophages and dendritic cells are responsible for the vasodilatation, increased vascular permeability and DIC of filoviral hemorrhagic fever. As described below, this work paid off with the recent demonstration that Ebola-infected macaques could be protected from death through blockade of the extrinsic coagulation pathway. By revealing an interlocking relationship among viral replication, inflammation and coagulation, this work gives reason to hope that a number of approaches could be used to prevent the development of hemorrhagic fever in humans who are infected with Marburg or Ebola virus, but have not yet become ill.

5.2.1 Viral suppression of type I IFN responses

Since most, if not all viruses have acquired mechanisms of blocking or evading IFN in the course of their evolution, it is reasonable to assume that the filoviruses inhibit these responses in their unidentified reservoir species, as a means of prolonging infection and increasing the likelihood of transmission to new hosts. One might therefore explain the extraordinary virulence of Marburg and Ebola virus for humans and nonhuman primates by proposing that their ability to suppress type I IFN responses proves “too successful” when the agents encounter the primate immune system, resulting in rapidly overwhelming infection (Bray, 2005).

Efforts to develop therapies that compensate for filovirus-induced suppression of type I IFN responses have so far been more successful in rodent models than in nonhuman primates. For example, pre- or postexposure prophylaxis of mice with the cross-species-active chimeric B/D form of human IFN- α prevents the development of illness after challenge with mouse-adapted Ebola Zaire virus (Bray, 2001). The IFN inducer polyICLC and synthetic oligodeoxynucleotides containing unmethylated CpG motifs, which stimulate macrophages to release IFN- α and other proinflammatory cytokines, are also effective for pre- or early postexposure prophylaxis in mice (Klinman *et al.*, 1999; Bray, 2001).

As noted, the induction of a strong type I IFN response is the mechanism by which the adenosine analogs c3-Npc A and C-c3Ado protect Ebola-infected mice. The quantity of circulating IFN induced by treatment increases progressively when the dose is delayed from day 0 to day 1 and

day 2. Since they do not induce IFN production in normal mice, these data indicate that these compounds exert their effect only in virus-infected cells (Bray *et al.*, 2000, 2002). A likely mechanism of action is that inhibition of cellular SAH hydrolase results in impaired mRNA cap methylation, causing the accumulation of untranslated viral mRNA transcripts, some of which may associate with negative-sense genomic strands to form dsRNA that acts as a strong stimulus for IFN production.

This previously unrecognized effect of SAH hydrolase inhibitors may help to explain their broad-spectrum antiviral activity. Efforts are now under way to determine whether single-dose therapy with c3-Npc A is also effective against other RNA virus infections in rodents. Understanding the mechanism by which adenosine analogs induce IFN production in virus-infected, but not in uninfected cells, and the identification of compounds that can achieve such an effect in primates, would represent a genuine breakthrough in antiviral therapy, since treatment could selectively eliminate foci of viral replication without causing the systemic signs and symptoms associated with IFN therapy.

Despite considerable evidence that filoviruses suppress type I IFN responses in primate cells, little effort has been made to evaluate IFN treatment or other countermeasures in nonhuman primates. In the only reported attempt at IFN therapy, four rhesus macaques were given large daily doses of recombinant human IFN- α 2b, beginning immediately after challenge with Ebola Zaire virus, resulting in a 1-2-day delay in the onset of illness, viremia and death (Jahrling *et al.*, 1999). Since natural IFN responses include IFN- β and multiple subtypes of IFN- α , it is evident that a variety of strategies, including the administration of a mixture of IFNs or the use of IFN inducers such as polyICLC or CpG, might improve on the results of this pilot study.

5.2.2 Modulation of inflammation/coagulation

The fever, shock and hemorrhage that characterize lethal filovirus infection are caused by inflammatory mediators released from virus-infected macrophages and other cells (Bray and Mahanty, 2003; Bray and Geisbert, 2005). In Ebola-infected cynomolgus macaques, these processes develop so quickly that the animals become ill within 3 days after virus challenge and die on day 6-8, a course too rapid to permit development of antigen-specific immune responses. In humans, by contrast, the process is slower, such that death usually occurs during the second week of illness, providing additional time for the development of adaptive responses required for survival. Limited data from Africa suggest that the outcome of illness may in fact be

determined soon after the entry of virus into the body, by the nature and timing of innate immune responses (Baize *et al.*, 1999, 2002). Taken together, these findings suggest that modulation of innate responses could slow viral dissemination and aid development of antigen-specific immunity.

A recent experiment demonstrated that such intervention can be surprisingly effective. Using the uniformly lethal model of Ebola Zaire virus infection in macaques, Geisbert *et al.*, treated 9 animals with daily doses of recombinant nematode anticoagulant factor C2 (rNAPc2), an inhibitor of TF-factor VIIa interaction, beginning either immediately after or the day following virus challenge (Geisbert *et al.*, 2003a). Even though rNAPc2 had no direct antiviral activity in cell culture, therapy was quite beneficial: three of the treated animals survived infection, while the average time to death of those that did become ill was significantly greater than that of placebo-treated animals. Treatment markedly reduced physical signs of coagulopathy and the levels of D-dimers and other markers of DIC in the blood, and also caused significant decreases in blood levels of the proinflammatory cytokines IL-6 and MCP-1. Mean peak circulating viral titers in surviving macaques were 100-fold lower than in the control group.

These wide-ranging effects of treatment with a substance that was only expected to block the extrinsic coagulation pathway have important implications for the development of effective immunomodulatory therapies. First, the results clearly demonstrate that host responses to filoviral replication are damaging, since triggering of the extrinsic coagulation pathway by infected macrophages in untreated macaques resulted in more severe disease than in animals in which that pathway was blocked. These findings also serve as a reminder that viral replication, inflammation and coagulation should not be thought of as independent processes. Instead, they form such a tightly interlinked matrix that the inhibition of coagulation can “feed back” to suppress the first two components. The mechanism of communication between the induction of coagulation and other macrophage activities may be signalling from the cytoplasmic tails of cell-surface TF molecules (Ruf, 2004). Since these responses begin with the first infected macrophage, it is obviously essential to characterize filovirus infections at that level.

Efforts to modify inflammatory responses in filovirus-infected animals have otherwise been limited to a series of small experiments performed in Russia, the results of which have yet to be confirmed by other investigators. Ignat'ev *et al.*, first reported that when Marburg-infected guinea pigs were treated with the compound desferoxamin (desferal), which allegedly blocks the induction of endothelial cell adhesion molecules by TNF- α , three of six animals survived infection (Ignat'ev *et al.*, 1996). Similarly, when five Marburg-infected guinea pigs were treated daily, beginning on day 3

postinfection, with anti-TNF- α serum, three of them survived, while all control animals died (Ignat'ev, 2000). The same group recently reported that treatment of Marburg-infected guinea pigs with the licensed anti-TNF- α mab, Remicade®, worsened the outcome if begun soon after virus challenge, by shortening the mean time to death, while therapy begun on day 3 led to 50% survival (Ignat'ev, Bukin, and Otrachevskaia, 2004). If confirmed in larger, placebo-controlled experiments, these results would be in agreement with observations from clinical trials in septic shock, which suggest that proinflammatory cytokine responses play a protective role early in infection, but are harmful if they continue for too long or are expressed in an unregulated manner (Hotchkiss and Karl, 2003).

5.2.3 Strategies employing genetically-engineered viruses

In contrast to Marburg and Ebola virus, the vast majority of microorganisms encountered by humans evoke innate responses that prevent the development of disease. The ability of the primate immune system to block most viral infections suggests that it might be possible to suppress a filovirus infection by “sending in” another microbe that induces protective responses in the same target cell population (Margolis, 2003). Current laboratory methods now make it possible to design an agent for this purpose.

An example of a genetically engineered virus that might be used in this manner is VSV, which is nonpathogenic for humans. A chimeric VSV, in which the GP gene is replaced by that of Ebola or Marburg virus, has proven highly effective as a conventional vaccine against these agents in laboratory animals (Garbutt *et al.*, 2004; Jones *et al.*, 2005). Since the vaccine virus displays a filoviral surface GP, it might also be effective if administered soon after filovirus infection, since the virions will attach to the same cell types targeted by the authentic pathogens, but their replication will evoke strong innate responses, including the production of large amounts of type I IFN, that could suppress filoviral replication in the same or neighbouring cells. Therapeutic efficacy could potentially be improved by engineering the gene encoding the VSV M protein to induce an enhanced IFN response (Stojdl *et al.*, 2003). Such a chimeric agent could thus act as both a drug and a vaccine, by inducing both immediate protection and long-term immunity in those who have been infected with a filovirus, but have not yet become ill.

6. TARGETS FOR FURTHER RESEARCH

The extreme virulence of Marburg and Ebola virus for humans poses a major challenge for the development of antiviral therapy. At present, it

appears that the most easily achievable goal is the development of an effective form of postexposure prophylaxis. Because early, rapid viral dissemination and many features of the subsequent illness result from interactions between filoviruses and macrophages and dendritic cells, it will be essential to obtain a detailed understanding of these events at the molecular level.

As regards the development of direct inhibitors of filovirus replication, it is not possible at present to identify the approach that is most likely to lead to a licensed medication. Since the level of viremia in Ebola hemorrhagic fever patients is a critical determinant of survival or death, it is clearly reasonable to pursue the development of antibodies and lectins that bind to virus in the bloodstream and prevent its spread to new target cells. Because it would also be highly desirable to inhibit the filoviral RNA-dependent RNA polymerase complex, determination of the structures of its components and their mode of interaction is also an important scientific objective. Such efforts should follow leads provided by studies of related negative-strand RNA viruses that are easier to manipulate in the laboratory. Those attempting to devise means of preventing filoviruses from exploiting host protein-processing pathways for entry into cells and the assembly and release of new virions should also monitor the success of efforts to employ such strategies against HIV and other pathogens.

As regards the indirect suppression of Marburg and Ebola virus replication through modulation of host responses, several approaches have shown promise. It will be essential to learn in detail how filoviruses suppress type I IFN responses and the role that such inhibition plays in disease. In particular, the identification of compounds that stimulate the production of IFN by virus-infected primate cells, in the manner of adenosine analogues in mice, could lead to major progress in the treatment of severe viral infections. The recent discovery that inhibition of the extrinsic coagulation pathway has widespread effects, including suppression of viral replication, should also be exploited for the development of new therapeutic approaches. By "correcting" damaging host responses, such interventions may prove beneficial even in the absence of specific antiviral therapy.

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