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MARBURG HAEMORRHAGIC FEVER: RECENT ADVANCES

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ABSTRACT
Viral hemorrhagic fevers (VHFs) are a group of etiologically diverse viral diseases unified by common underlying pathophysiology. These febrile diseases result from infection by viruses from four viral families: Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae. The viruses in the four families are all RNA viruses. All share the feature of having a lipid envelope. Survival and perpetuation of the viruses is dependent on an animal host known as a natural reservoir, but humans are not the natural reservoir. With the exception of a vaccine for yellow fever and ribavirin, which is used for treatment of some arenaviral infections, no specific chemotherapy for viral hemorrhagic fever exists. Only supportive treatment is possible The filoviruses, Marburg virus (MARV) and Ebola virus (EBOV), have been associated with hemorrhagic fever (HF) that produce severe disease and high mortality rates among infected humans and non-human primates. MARV and EBOV are also considered potential biological weapons. Although much progress has been made in developing preventive vaccines and postexposure interventions that can protect laboratory animals and nonhuman primates against lethal challenge with MARV, none of these has been approved for humans. Because MARV haemorrhagic fever, when it occurs, has the potential to spread to other people especially health care staff and family members who care for the patient, there is need for periodic review of recent developments relating especially to its diagnosis and treatment. This would help to increase awareness among health-care providers and limit the spread of the disease during outbreaks.

Keywords: Marburg virus, viral haemorrhagic fever, recent advances

Background

Viral hemorrhagic fevers (VHFs) are a group of etiologically diverse viral diseases unified by common underlying pathophysiology. These febrile diseases result from infection by viruses from four viral families: Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae. The viruses in the four families are all RNA viruses. All share the feature of having a lipid envelope. Survival and perpetuation of the viruses is dependent on an animal host known as a natural reservoir, but humans are not the natural reservoir. With the exception of a vaccine for yellow fever and ribavirin, which is used for treatment of some arenaviral infections, no specific chemotherapy for viral hemorrhagic fever exists. Only supportive treatment is possible (1).

The filoviruses, Marburg virus (MARV) and Ebola virus (EBOV), have been associated with hemorrhagic fever (HF) that produce severe disease and high mortality rates among infected humans and non-human primates (2). MARV and EBOV are also considered potential biological weapons. Although much progress has been made in developing preventive vaccines and postexposure interventions that can protect laboratory animals and nonhuman

primates against lethal challenge with MARV, none of these has been approved for humans (3).

History

MARV was first identified during simultaneous outbreaks in 1967 when infected monkeys, imported from the Lake Kyoga region of Uganda, transmitted the virus to laboratory workers and scientists at facilities in Marburg and Frankfurt, Germany and Belgrade in the former Yugoslavia (3,4). The persons affected had contact with the blood or tissues of monkeys or with other infected persons. Other Marburg haemorrhagic fever epidemics which have occurred since then include one from October 1998 through September 2000 in Durba, Democratic Republic of the Congo (5). The outbreak involved 154 patients (48 confirmed and 106 suspected cases); the case fatality ratio was 83% (6).

In March 2005, the Centers for Disease Control and Prevention (CDC) investigated a large HF outbreak in Uige Province in northern Angola, West Africa. In total, 15 initial specimens were sent to CDC, Atlanta, for testing for viruses associated with viral HFs known to be present in West Africa, including Ebola virus. Marburg virus was also included despite the fact that the origins of all earlier outbreaks were linked directly to East Africa. Surprisingly, Marburg virus was confirmed (12 of 15 specimens) as the cause of the outbreak. The outbreak likely began in October 2004 and ended in July 2005, and it included 252 cases and 227 (90%)

fatalities (report from the Ministry of Health, Republic of Angola, 2005), making it the largest Marburg HF outbreak on record (7). Two smaller outbreaks occurred in 2007 and 2008 in Uganda and the Netherlands respectively. The outbreak in Uganda involved 2 cases, one fatal, in young males working in Lead and gold mine in Kamwenge District, Uganda. The latter case, which was fatal, involved a 40-year-old Dutch woman in the Netherlands with a recent history of travel to the Python Cave, Uganda (3). On February 9, 2009, it was reported that in January 2008, a US Citizen from Colorado was the first patient treated in the United States for Marburg. The patient had contracted the virus while overseas in Uganda and traveled back to the USA, where she was later treated successfully for the infection (8).

Aetiology

Marburg virus, or simply Marburg, is the common name for the genus Marburgvirus which contains one species: Lake Victoria marburgvirus. The virus causes the disease Marburg Hemorrhagic Fever (MHF), also referred to as Marburg Virus Disease, and previously also known as Green Monkey Disease due to its primate origin. Marburg originated in Central and East Africa, and infects both human and nonhuman primates. The Marburg Virus is in the same taxonomic family as Ebola, and both are identical structurally although they elicit different antibodies (8). The two viruses comprise the family Filoviridae, order Mononegavirales (Peters and Khan, 1999 9). MARV is a single species consisting of viruses differing from one another by up to 21% at the nucleotide level. For instance, during the epidemics which occurred from 1998 to 2000 in the Democratic Republic of Congo, at least nine genetically distinct lineages of the virus were in circulation (6).

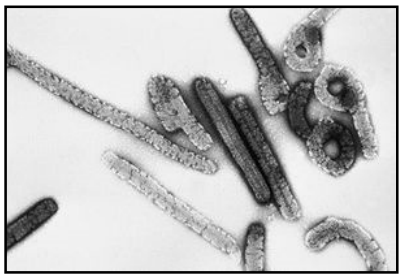

Plate 1: Marburg virus particles (Appro ximately 100,000x magnification). Adapted from species.wikimedia.org/wikifiloviridae

In contrast, four distinct species of ebolavirus (Zaire, Sudan, Reston, and Ivory Coast) have been defined, which differ genetically from one another by approximately 37 to 41% (9). The structure of MARV is typical of filoviruses, with long threadlike particles which have a consistent diameter but vary greatly in length from an average of 800 to 14,000 nanometers (nm), with peak infectious activity at about 790 nm (Plate 1). Marburg virus contains a single molecule of linear negative-sense, 19.1 kb single-stranded RNA whose seven gene products are, in order, nucleoprotein (NP), VP35, VP40, glycoprotein (GP), VP30, VP24, and the polymerase (L) (10).

Epidemiology and Ecology of Marburg Virus Haemorrhagic Fever

Outbreaks of Marburg are centered in Africa, where the natural reservoir is believed to be located. Historically, sources of MARV were confined to East Africa. They had been centered almost exclusively within 500 miles of Lake Victoria, with the exception of a single case in Zimbabwe in 1975, when a traveler became infected and seeking medical treatment, subsequently transmitted the virus to a health care worker in South Africa. This previous close association of MARV with East Africa contrasts with the observed distribution of EBOV,which has caused human HF outbreaks throughout tropical Africa, ranging from Coted'Ivoire to Uganda. However, a large MARV HF outbreak occurred in Uige Province in northern Angola, West Africa in 2005 (7).

MARV and EBOV HF outbreaks are generally thought to involve the relatively rare introduction of the virus into the human population followed by waves of human-to-human transmission (usually through close contact with infected individuals or their body fluids) (31). Although the environmental reservoir of MARV was previously unknown (CDC, 2005 11), in a study carried out to determine reservoir hosts for MARV in Democratic Republic of the Congo, the fauna of a mine which was associated with a protracted outbreak of Marburg hemorrhagic fever during 1998 to 2000 were examined and MARV nucleic acid was found in 12 bats, comprising of two species of insectivorous bat and one species of fruit bat. Antibodies to the virus were also detected in the serum of some of the insectivorous and fruit bat species, but attempts to isolate virus were unsuccessful (17). Pourrut *et al*.,2009 (30) also suggested, based on results of their studies, that the bat species *Rousettus aegyptiacus* may be involved in the natural cycle of both Marburg and Ebola viruses.

Transmission and Pathogenesis of Marburg Haemorrhagic Fever

Marburg virus is transmitted by direct contact with the blood, body fluids and tissues of infected persons. Transmission of the Marburg virus also occurred by handling ill or dead infected wild animals (monkeys, fruit bats) (3). After gaining access to the body, filoviruses initially infect monocytes, macrophages and other cells of the mononuclear phagocytic system (MPS), probably in regional lymph nodes. Some infected MPS cells migrate to other tissues, while virions released into

the lymph or bloodstream infect fixed and mobile macrophages in the liver, spleen and other tissues throughout the body. Virions released from these MPS cells proceed to infect neighboring cells, including hepatocytes, adrenal cortical cells and fibroblasts (12).
Infected MPS cells become activated and release large quantities of cytokines and chemokines, including TNF-, which increases the permeability of the endothelial lining of blood vessels. Endothelial cells apparently become infected by virus only in the later stages of disease. Circulating cytokines contribute to the development of disseminated intravascular coagulation (DIC) by inducing expression of endothelial cell-surface adhesion and procoagulant molecules and tissue destruction results in the exposure of collagen in the lining of blood vessels and the release of tissue factor (12). Massive lysis of lymphocytes occurs in the spleen, thymus and lymph nodes in the late stages of filovirus infection. There is no sign that the lymphocytes themselves are infected, rather they die through apoptosis, perhaps induced by cell-surface binding of chemical mediators released by MPS cells or by a viral protein. Massive cytolysis, immune dysfunction, fluid shifts, microvascular coagulation and interstitial hemorrhage all play a role in the development of shock and death (12).

Clinical Signs and Prognosis of Marburg Haemorrhagic Fever

Filovirus infections, in general, are the most severe of the viral hemorrhagic fevers. After an incubation period of 4 to 10 days, to a maximum of three weeks (13Jeffs, 2006), infected individuals abruptly develop flu-like symptoms characterized by fever, chills, malaise, and myalgia. Approximately the fifth day after onset of symptoms, a maculopapular rash might occur, after which patients usually develop other signs and symptoms that indicate systemic involvement, such as prostration and gastrointestinal (anorexia, nausea, vomiting, abdominal pain, and diarrhea), respiratory (chest pain, shortness of breath, and cough), vascular (conjunctival injection, postural hypotension, and edema), and neurological (headache, confusion, delirium and coma) manifestations (11).

The target organ in the VHF syndrome is the vascular bed; correspondingly, the dominant clinical features are usually a consequence of microvascular damage and changes in vascular permeability. Bleeding is manifested as petechiae, ecchymosis, uncontrolled oozing from venipuncture sites and gingiva, mucosal hemorrhages, and bloody diarrhea. In later stages, the general condition of patients deteriorates due to multiorgan failure, including disseminated intravascular coagulopathy, resulting in death (11,14). If a patient survives, recovery is usually prompt and complete, though it may be prolonged in some cases, with inflammation or secondary infection of various organs, including: orchitis, hepatitis, transverse myelitis, uveitis, and parotitis. Recovered patients often have little or no memory of being sick, though only 10-40% survive (16). Case fatality rates of Marburg haemorrhagic fever have varied greatly, from 25% in the initial laboratory-associated outbreak in 1967, to more than 80% in the Democratic Republic of Congo from 1998-2000, to even higher in the outbreak that began in Angola in late 2004 (3,11).

Prevention of Marburg Virus Infection

MARV is a biosafety level-four agent (BSL-4), and thus requires the highest level of precautions (18). While over-reaction on the part of medical personnel is inappropriate and detrimental to both patient and staff, it is prudent to provide isolation measures as rigorous as feasible. At a minimum, these should include the following: stringent barrier nursing; mask, gown, glove, and needle precautions; hazard-labeling of specimens submitted to the clinical laboratory; restricted access to the patient; and autoclaving or liberal disinfection of contaminated materials, using hypochlorite or phenolic disinfectants (MARV is susceptible to 1% sodium hypochlorite, 2% gluteraldehyde or formaldehyde, ultraviolet light and heat). For more intensive care, however, increased precautions are advisable. Members of the patient care team should be limited to a small number of selected, trained individuals, and special care should be directed toward eliminating all parenteral exposures. Use of endoscopy, respirators, arterial catheters, routine blood sampling, and extensive laboratory analysis increase opportunities for aerosol dissemination of infectious blood and body fluids.

A few research groups are working on vaccines to fight the virus. In 1998, a group at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) published the first peer reviewed article detailing the development of the first experimental Marburg virus vaccine demonstrated to completely protect animals from lethal Marburg virus infection (19Hevey *et al.*, 1998). Following this, in 2002, Genphar, a company doing research for the United States Army's biodefense program, announced that an experimental vaccine protected animals from a high dose of Marburg virus. The tests were conducted by USAMRIID. According to the company, all animals in the control group died within days whereas all animals that received the regular dosage of the vaccine were fully protected (8).

Post-exposure Treatment of Marburg Virus Infection

There is no specific antiviral therapy indicated for treating Marburg, and hospital care is usually supportive in nature. Hypotension and shock may

require early administration of vasopressors and haemodynamic monitoring with attention to fluid and electrolyte balance, circulatory volume, and blood pressure. Viral haemorrhagic fever (VHF) patients tend to respond poorly to fluid infusions and may develop pulmonary edema. (3,8). However, several attempts have been made to develop postexposure interventions against the filoviruses. Some degree of success has been achieved by using strategies that mitigate the coagulation abnormalities characterizing filoviral infection (20,28). Also, new postexposure treatment approaches, based on small interfering RNA (21) and antisense oligomers (22), have shown promising results in rodent models, but no reports have been published of evaluations of either strategy in the more stringent macaque models.

In 2006, the first complete postexposure protection of nonhuman primates against a filovirus was reported. This was done by administering a live-attenuated recombinant vesicular stomatitis virus (rVSV) vaccine vector expressing the MBGV glycoprotein (GP) (VSVΔG MBGV GP) shortly after a high-dose MBGV challenge (27). In a follow-up study to the one above, rhesus monkeys were protected from MARV disease when a recombinant vesicular stomatitis virus–based vaccine was administered 20 to 30 minutes after infection with Marburg virus. Five out of six (5/6) monkeys were protected when this vaccine was given 24 h after challenge, while 2/6 animals were protected when the vaccine was administered 48 h postinfection (22).

More recently, results obtained from studies conducted by the U.S. Army Medical Research Institute of Infectious Diseases in collaboration with AVI BioPharma, a Washington-based biotechnology firm, have remained very promising for post-exposure treatment of MARV infection. Their studies show that novel antisense therapies targeting specific viral genes protected monkeys infected with deadly Ebola or Marburg viruses, even when therapeutics were administered one hour after exposure—suggesting the approach holds promise for treating accidental infections in laboratory or hospital settings (23).

Diagnosis of Marburg Virus Infection

It should be kept in mind that the diagnosis of MARV infections will initially have to be based on clinical assessment (24). Clinicians should consider the diagnosis of Marburg VHF among febrile patients who, within 10 days before onset of fever, have either 1) traveled in northern Angola; 2) had direct contact with blood, other body fluids, secretions, or excretions of a person or animal suspected of having VHF; or 3) worked in a laboratory or animal facility that handles hemorrhagic fever viruses. The likelihood of acquiring VHF is considered extremely low in persons who do not meet any of these criteria. The cause of fever in persons who have traveled to areas where VHF is endemic is more likely to be a different infectious disease (11). When the identity of a VHF agent is totally unknown, isolation in cell culture and direct visualization by electron microscopy, followed by immunological identification by immunohistochemical techniques is often successful. Immunohistochemical techniques are also useful for retrospective diagnosis using formalin-fixed tissues, where viral antigens can be detected and identified using batteries of specific immune sera and monoclonal antibodies (24).

Formal laboratory diagnosis requires a laboratory with special containment facilities (BL-4 containment) (24). Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing, IgM-capture ELISA, polymerase chain reaction (PCR), and virus isolation can be used to confirm a case of Marburg hemorrhagic fever within a few days of the onset of symptoms. The IgG-capture ELISA is appropriate for testing persons later in the course of disease or after recovery. The disease is readily diagnosed by immunohistochemistry, virus isolation, or PCR of blood or tissue specimens from deceased patients (13, 14). MARV grows well in a large variety of cell lines, although Vero or Vero E6 cells have been most used. The virus is relatively stable and may survive unfavorable handling and shipping (15 Sanchez *et al.*, 2001). Diagnosis by viral cultivation and identification requires 3 to 10 days. However, viral isolation should not be attempted without BL-4 containment (24).

Weaponization and Bioterrorism

The viral hemorrhagic fever (VHF) agents, including MARV, are all highly infectious via the aerosol route, and most are quite stable as respirable aerosols. This means that they satisfy at least one criterion for being weaponized, and some clearly have the potential to be biological warfare threats (24). The former Soviet Union reportedly had a large biological weapons program involving Marburg. They developed a new strain, called "Variant U," which was successfully weaponized and approved by Soviet Ministry of Defense in 1990 (25). Bioterrorism grants in the United States are funding research to develop a vaccine for Marburg virus (8).

Conclusion

Marburg hemorrhagic fever is a very rare human disease. However, when it occurs, it has the potential to spread to other people, especially health care staff and family members who care for the patient. Increasing awareness, among health-care providers, of clinical symptoms in patients that suggest Marburg hemorrhagic fever is therefore

critical for limiting the spread of the disease during outbreaks.

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