Viral Disease in Hematology

Genoveffa Franchini (Chair), Richard F. Ambinder, and Michèle Barry

As part of the international outreach of the American Society of Hematology, this review addresses some aspects of the genetics, biology, epidemiology, and clinical relevance of viruses that cause a variety of hematopoietic disorders in human populations. The viruses described here have a different pattern of geographical distribution, and the disease manifestations may vary according to environmental and/or genetic characteristics of the host. Epstein-Barr virus, a linear double-stranded DNA virus (herpesvirus), and the human T-cell leukemia virus, a retrovirus with a single-stranded diploid RNA genome, are associated among other diseases with lymphoma and leukemia/lymphoma, respectively. Both viruses cause a lifelong infection, but only a small percentage of infected individuals develop hematopoietic neoplasms. Epidemiological data suggest that the time of infection may be important in determining disease outcome in both HTLV-I and EBV infection. The pathogenic mechanisms used by these viruses are of most interest since they may recapitulate growth dysregulation steps also occurring in other hematopoietic malignancies.

In Section I Dr. Franchini reviews the biology, genetics and diseases associated with HTLV-I and HTLV-II. In Section II, Dr. Ambinder reviews the biology of EBV infection and its relationship to the pathogenesis of Hodgkin’s disease and other malignancies.

In Section III, Dr. Barry reviews the viral hemorrhagic fevers caused by RNA viruses such as Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae, which can lead to acute syndromes that can be fatal. However, prompt diagnosis is key for patient management as well as for limiting their spread to others. These syndromes have become the focus of public concern and represent not only a clinical challenge, since in most cases no specific antiviral treatment is available, but also a challenge for future basic research on their biology and pathogenesis since little is known at present.

I. Disease Association, Genetics, and Biology of Human T-cell Lymphotropic Virus Types I and II

Genoveffa Franchini, M.D.*

Discovery and Classification

Twenty-three years ago, Takatzuki and his collaborators discovered an unusual cluster of adult T cell leukemia/lymphoma (ATLL), which suggested the possible involvement of a transmissible agent in the disease.1 The first description of HTLV-I immediately followed the discovery of the human T cell growth factor (TGF, now called interleukin-2)2 that allowed long-term in vitro culture of T cells and the establishment of T cell lines from a patient in the U.S. with a cutaneous T cell lymphoma, who harbored type C retroviral particles. Simultaneously, a cell line derived from a patient with ATLL in Japan was also shown to harbor a retrovirus and to produce antigens that reacted with sera from patients with ATLL.3 This virus, designated HTLV-I, was identified as the etiological agent of ATLL.4 HTLV-I is not only the etiological agent of a human cancer but also associated with a progressive myelopathy designated HTLV-I-associated myelopathy or tropical spastic paraparesis (TSP/HAM).5,6 HTLV-II, originally isolated from a patient with an atypical form of T cell leukemia has so far not been associated morphologically with human diseases.

Classification

HTLV-I belongs to a distinct group of the subfamily of Oncovirinae. Several other retroviruses, including human T cell lymphotropic virus type II (HTLV-II), simian T cell lymphotropic virus types I and II (STLV-I and STLV-II), and the more distantly related bovine leukemia virus (BLV), have been classified in this group, based upon genetic sequence and structural homologies (reviewed in

* Section of Animal Models and Retroviral Vaccines, Basic Research Laboratory, Division of Basic Sciences, National Cancer Institute, 41 Library Drive, Room D804, Bethesda MD 20892
These exogenous viruses do not carry a known oncogene in their proviral genome and integrate randomly into the host cellular DNA. Therefore, in contrast to the two recognized mechanisms of retroviral oncogenesis (cis-activation of a cellular oncogene associated with a specific integration site in the host genome or transduction of a cellular oncogene that becomes part of the viral genome), viruses in the HTLV family use different strategies to induce neoplastic transformation. In addition to genes that encode for viral structural proteins, these viruses possess in the 3’ end of their genome open reading frames encoding for regulatory proteins (Tax, Rex, p21rex, p12, p13, and p30) that are involved in viral replication and/or pathogenesis. All these viruses display different patterns of geographical distribution throughout the world.

**Viral Transmission and Diagnosis of HTLV-I Infection**

Breast-feeding from mother to child is the primary method of HTLV-I transmission. Another route of transmission is through blood or blood-cell products, but, unlike the human immunodeficiency virus (HIV), cell-free blood-derived products are not infectious. Sexual transmission occurs more effectively from men to women via virus-infected cells present in the semen; transmission from women to men is rare. The diagnosis of HTLV-I infection is normally determined by Western blot analysis of sera from individuals suspected to carry the virus. Immunoreactivity to both the viral envelope and gag protein is a criteria of positivity. Differentiation between HTLV-I and HTLV-II infections needs to be accomplished since the long-term consequences of infection with the type I or the type II virus differ.

**Epidemiology**

HTLV-I is endemic in areas of southern Japan, the Caribbean basin, Africa, and eastern parts of South America. Molecular analysis has shown that HTLV and STLV originated from an ancestor virus that may have been transmitted to humans by contact with nonhuman primates (reviewed in 9). It was recently demonstrated by PCR amplification of HTLV-I proviral sequences from Andean mummies that HTLV-I has been present in humans for a long period of time.10 The presence of highly conserved viral strains in different geographic locations supports the hypothesis that the slave trade contributed to disseminating HTLV-I. An estimated 10 to 20 million people worldwide are infected with the virus. Most of these individuals remain asymptomatic carriers, but a small fraction, 1 to 4%, develop ATLL, usually many decades after the primary infection. The occurrence of ATLL is epidemiologically associated with HTLV-I infection at birth. The low incidence and long latency of HTLV-I-associated ATLL suggest that, in addition to viral infection, the accumulation of other genetic mutations may be needed for induction of ATLL. TSP/HAM, a neurological disorder, is the other major disease associated with HTLV-I infection.6,11 Generally, this disease evolves chronically and progressively although some aggressive forms of TSP/HAM have been reported after transfusion with blood from HTLV-I-infected donors.

**Viral Tropism**

Different from the well-defined HTLV-I tropism in vivo, which is mainly restricted to the CD4+ helper T-cell subset, HTLV-I also infects a wide variety of human and nonhuman cell types in vitro. Because cell-free virus preparations are poorly infectious, infection in vitro is generally obtained by cocultivation of the target cells with HTLV-I-producing cell lines. Interference between HTLV-I and HTLV-II infection indicates that the viruses share a common cell-surface receptor. Several strategies using binding, syncytial, and pseudotype assays suggest that the HTLV-I receptor is ubiquitously expressed and has been assigned to human chromosome 17. This receptor’s identity is unknown.

**HTLV-I-Associated Pathologies**

As established by epidemiological studies, HTLV-I is the etiological agent of ATLL (an aggressive lymphoproliferative disease) and TSP/HAM (an inflammatory neurodegenerative disorder). Other diseases, such as chronic arthropathy, uveitis, infective dermatitis, and polymyositis, have been associated with HTLV-I infection. As no difference between ATLL- and TSP-derived viruses by sequence or LTR-controlled transcription assay has been identified, the events that lead to the occurrence of hematological versus neurological disease are unknown.

ATLL, first described in Japan in 1977, presents as an oligoclonal or monoclonal expansion of virus-infected T cells that occurs many decades after infection by HTLV-I. The presence in the blood of flower-like T cells of a mature activated phenotype (CD2+, CD3+, CD4+, CD8-, CD25+, and HLA-DR+ cellular surface markers) is considered pathognomonic of HTLV-I infection. ATLL encompasses a large clinical spectrum with a broad pattern of symptoms. Some infected individuals develop a pre-ATLL syndrome that is mainly characterized by a lymphocytosis resulting from proliferation of a few clones of HTLV-I-infected cells. Approximately half of these individuals experience spontaneous regression. Some infected individuals develop a smoldering or chronic form of ATLL. The chronic stage presents with lymphocytosis, and patients generally suffer from adenopathy and splenomegaly. Smoldering ATLL is characterized by a low level of circulating virus-infected cells and, at times,
skin lesions that are caused by infiltration of leukemic cells. Some individuals eventually progress to an acute stage of ATLL, an aggressive form of leukemia. Acute ATLL is characterized by monoclonal expansion of virus-infected T cells that can ultimately represent up to 99% of the total white blood cell population. Patients exhibit skin lesions and present with polyadenopathy and hepatosplenomegaly. Hypercalcemia is frequent and is an important clinical challenge. Finally, some infected individuals develop clonal T cell lymphomas that contain integrated HTLV-I proviruses. These patients present with a low percentage of circulating leukemic cells and polyadenopathy. Patients with acute ATLL or HTLV-I-associated lymphomas have a life expectancy of approximately 6 to 10 months.

Genetic Organization
The genetic organization of HTLV-I is more complex than that of other animal onco-retroviruses and, in addition to structural and enzymatic proteins, the proviral genome encodes regulatory proteins like Tax and Rex. The HTLV-I long terminal repeat (LTR) is divided into three regions: U3, R, and U5. The U3 region contains regulatory elements that control viral transcription. In addition to the gag (core), pol (polymerase and reverse transcriptase interpose and integrase), and the env genes, HTLV-I carries a region at its 3' end with the potential to encode more proteins. The well-characterized regulatory proteins Tax and Rex are encoded by open reading frames (ORF) IV and III, respectively, and several other genes, p12I, p13II, p30II, are encoded by ORF I and II. Expression of these mRNAs has been detected in cells infected by HTLV-I in vitro and in vivo samples isolated from asymptomatic carriers and ATLL and TSP/HAM patients.

Tax, a 40 kDa nuclear phosphoprotein, interacts with members of the ATF/CREB, NF-6B family of transcription factors and activates or represses transcription. Tax also activates transcription from the serum-responsive elements; however, little is known about the molecular mechanisms involved.

In addition to its trans-activating functions, HTLV-I Tax affects cell-cycle regulatory proteins, resulting in dysregulated growth of infected T cells (reviewed in 12). Cyclin-dependent kinases (CDK) CDK4 and CDK6 can form complexes with D-type cyclins (D1, D2, and D3) to promote phosphorylation of the retinoblastoma tumor suppressor protein (Rb), releasing E2F and stimulating G1 to S progression of the cell cycle. In resting cells, CDK4 and CDK6 kinase activity is countered by specific inhibitors, termed INK4 proteins (p16INK4A, p15INK4B, p18INK4C, and p19INK4D). Tax binds and interferes with the ability of p16INK4A to repress CDK4 activity. Tax also complexes with cyclin D3, resulting in an increased CDK4/6 activity and hyperphosphorylation of Rb in the absence of p16INK4A expression. However, unlike other cells, expression of the CDK inhibitor p21WAF1/CIP1 is upregulated in HTLV-I-infected cells and decreases in response to antiproliferative signals. Additionally, decreased expression of p27KIP1 after HTLV-I infection coincides with constitutive activation of cyclin E-CDK2 complex and IL-2-independent proliferation. Another cell-cycle checkpoint, the tumor-suppressor p53 protein is targeted by Tax. Generally, p53 expression is induced upon DNA damage, resulting in G1 arrest or apoptosis induction. In Tax-expressing cells, although p53 expression is increased, p53 transcriptional activity is impaired. Finally, in addition to multiple targets of the G1/S transition control, Tax affects the G2/M checkpoint mitotic arrest defective (MAD1), possibly resulting in perpetuation of karyotypic abnormalities.

Rex is a 27 kDa phosphoprotein encoded by ORF III that plays an essential role in viral replication and the regulation of viral structural genes by functioning as a post-transcriptional regulator.

p13II/p30II/p12I
p13II and p30II are proteins encoded by the ORF-II reading frame. p13II localizes to the mitochondria and p30II to the nucleus. At present, little is known about their function.

p12I is a small hydrophobic protein encoded by ORF I, which localizes to cellular endomembranes. p12I has some structural similarity to the bovine papillomavirus type I (BPV-1) E5 oncoprotein and can enhance mouse fibroblast transformation mediated by E5. p12I binds to the β and (γ chains of the IL-2 receptor in vitro and increases STAT5b activation. In addition, p12I was shown to bind to the MHC Class I heavy chain in vitro and to prevent its association with β2-microglobulin (J.M. Johnson et al, submitted). As a result, the MHC I-Hc/p12I complex is in part targeted for degradation by the proteasome. This event may affect the level of MHC Class I expression on the cell surface and/or interfere with antigen presentation and contribute to HTLV-I escape from the host immune response.

Pathogenesis
HTLV-I-mediated T-cell transformation presumably arises from a multistep oncogenic process in which HTLV-I induces chronic T cell proliferation resulting in an accumulation of genetic defects and dysregulated growth of infected cells. Evidence suggests that Tax is intimately involved in this process. Tax-transactivating functions result in the increased expression of numerous cellular genes that presumably have an important effect on lymphomagenesis. Tax is able to immortalize primary T cells and transform fibroblasts in vitro. In addition, Tax induces the development of tumors in transgenic mice. It
is unclear which of the diverse functions of Tax are necessary for T cell immortalization. Some reports indicate that activation of the NF-κB pathway is sufficient while other reports demonstrate that the ATF/CREB pathway is responsible for Tax’s effect. However, it is likely that more than one of Tax’s functions is involved in initiating the immortalization process. Tax interferes with the DNA repair machinery and increases the mutation rate in virus-infected cells. Finally, Tax can antagonize or stimulate the apoptotic pathways under different growth conditions. Expression of anti-apoptotic Bcl-2 and Bcl-XL is upregulated in HTLV-I-expressing cells and the latter is also found in leukemic cells from ATLL patients. In this respect, Tax bears resemblance to other cellular and viral oncogenes (for example, c-myc, c-jun, E1A 12S, polyoma virus T antigen, and E7 proteins) that possess both transforming and apoptosis-modulating properties. By uncovering the molecular mechanisms underlying Tax leukemogenesis, we may gain more insight on the identity of cellular genetic alterations preceding the establishment of ATLL.

**Treatment of ATLL**

Conventional combination chemotherapy treatment of ATLL is used in the clinic. In the last few years, other approaches have also been used, including a combination of alpha interferon and zidovudine; allogeneic bone marrow transplantation; Yttrium-90-labeled anti-Tac (CD25, IL-2R chain) antibodies; and recombinant immunotoxins. However, in most patients, none of these treatments results in long-lasting remission.

**II. EPSTEIN-BARR VIRUS AND HODGKIN’S DISEASE**

*Richard F. Ambinder, M.D., Ph.D.*

The discovery of Epstein-Barr virus (EBV) infection in Reed-Sternberg cells in many cases of Hodgkin’s disease (HD) suggested the possibility that the relationship between the virus and the tumor might be a straightforward one. The idea that HD in young adults might be a rare consequence of a common infection had been suggested by variation in the bimodal age incidence curve of Hodgkin’s disease in association with the level of economic development. Evidence emerged that factors that influenced the timing of exposure to infection also influenced the risk of Hodgkin’s disease. Small family size and low housing density predicted an increased risk of Hodgkin’s disease and might be viewed as surrogates for delayed exposure to infection by a ubiquitous infectious agent. Hodgkin’s disease was specifically likened to the paralytic consequences of poliovirus infection which followed infection by poliovirus more commonly when infection occurred later in adolescence or adulthood rather than in infancy or childhood. Several sorts of observations supported the idea that EBV infection might be specifically linked. Seroepidemiologic studies showed titers to EBV antigens elevated both at the time of diagnosis of HD and several years in anticipation of diagnosis. A history of infectious mononucleosis was associated with increased risk of Hodgkin’s disease. In case reports Hodgkin’s disease developed in close association with primary EBV infection. However, the relationship between the virus and the malignancy has proven to be anything but straightforward.

**EBV**

EBV is a herpesvirus with a large genome of linear double-stranded DNA. Like other herpesviruses, infections are lifelong. The virus may be either lytic or latent. By lytic infection is meant productive infection yielding new virions, while latent infection implies persistence of the viral genome without the production of new virions. As with other human herpesviruses with the exception of the Kaposi’s sarcoma herpesvirus, EBV is ubiquitous with the great majority of the world’s adult population infected. Primary infection is usually asymptomatic in childhood but is commonly associated with the syndrome of infectious mononucleosis when it occurs in young adults.

EBV is distinguished from other common human herpesviruses such as herpes simplex and varicella-zoster virus by its establishment of latency in cells with the capacity to proliferate. The virus is transmitted in the saliva but spreads to B cells. These are driven to proliferate, replicating the viral genome in tandem with the cellular genome, and thus infection spreads throughout the B cell compartment. In time the cellular immune response targets cytotoxic T lymphocytes to viral antigen-expressing B cells. A subset of virus-infected cells with very limited viral antigen expression escape this immune surveillance and persist for life.

**Virus Expression and T Cell Responses**

In immortalized lymphocytes in vitro, EBV expresses six nuclear proteins, three membrane proteins, and the EBERs. EB nuclear antigen 1 (EBNA1) is required for maintenance of the viral episome. EBNA2 is a transcriptional transactivator, which turns on expression of a variety of viral and cellular genes important in regulating cell growth. Latency membrane protein 1 (LMP1) resembles a constitutively activated member of the tumor necrosis factor receptor (TNFR) superfamily and interacts with TNFR-associated proteins (TRAFs) that lead to activation of NF-κB and modulation of a variety of
apoptotic and growth pathways. LMP1 expression in immortalized murine cell lines leads to tumorigenicity in nude mice. The EBERs are short polymerase III transcripts whose function remains unknown.

The antigens most frequently targeted by cytotoxic T cells in healthy volunteers are EBNA 3A, 3B and 3C.\textsuperscript{7} Other latency antigens, including LMP1 and LMP2, are sometimes targeted. A glycine-alanine repetitive sequence within EBNA1 inhibits its antigen processing and MHC class I presentation. However, EBNA1 is universally recognized in MHC class II by CD4+ T cells.\textsuperscript{9}

**EBV(+) Tumors Other Than HD and Animal Models**

EBV is associated with various lymphomas, carcinomas, and even mesenchymal neoplasms.\textsuperscript{10} B cell lymphomas associated with EBV include Burkitt lymphoma, lymphoproliferations arising in patients with acquired or congenital immunodeficiencies, and pulmonary lymphomatoid granulomatosis.\textsuperscript{11,12} EBV is associated with virtually all undifferentiated nasopharyngeal and gastric carcinomas, as well as smooth muscle neoplasms occurring in immunocompromised patients.\textsuperscript{13-15} Some peripheral T cell or NK cell lymphomas have been associated with EBV, particularly nasal lymphoma.\textsuperscript{16}

Some of the B cell lymphoproliferative disease and lymphomas arising in organ transplant patients and AIDS patients resemble EBV-immortalized lymphoblastoid cell lines in terms of the pattern of viral gene expression, i.e. many latency genes are expressed. In nasopharyngeal carcinoma, gastric carcinoma, African Burkitt’s lymphoma, and nasal lymphoma there is a much more restricted pattern of viral gene expression. In particular these tumors do not express the immunodominant EBNAs (EBNAs 2, 3A, 3B, 3C).

Several animal models of EBV and tumorigenesis have been developed.\textsuperscript{17} Inoculation of cotton top marmosets leads to polyclonal B cell lymphoproliferative disease. Transfer of peripheral blood mononuclear cells from EBV-seropositive donors into mice with severe combined immunodeficiency (SCID) leads to the development of EBV-associated B cell tumors. Similarly, transfer of peripheral blood mononuclear cells from EBV-seronegative donors followed by infection with EBV, or transfer of EBV-immortalized B cell lines (i.e. infection in the mouse or infection in vitro before transfer to the mouse) leads to tumors derived from human B cells. All of the tumors arising in these models roughly resemble EBV-immortalized lymphoblastoid cell lines in their patterns of gene expression.

**EBV in Hodgkin’s Disease**

Several Southern blot hybridization studies showed the presence of EBV in some HD tumors and showed that the viral genome was clonal when present.\textsuperscript{18-20} In situ hybridization studies and antigen detection studies localized the virus to Reed-Sternberg cells and their variants in some tumors.\textsuperscript{21,22} EBNA1, LMP1, LMP2 and the EBERs are expressed in the Reed-Sternberg cells in approximately 30–50% of Hodgkin’s disease cases in Europe and North America.\textsuperscript{23-25} Detection of any of these targets yields nearly identical results. In general, lytic transcripts are not expressed.

Histologic type, age, sex, and ethnicity all affect the association of EBV with HD. A compilation of data on 1,546 HD patients from 14 studies examined the risk for EBV-positive disease.\textsuperscript{25} Odds ratios for EBV-associated HD were increased for mixed cellularity vs. nodular sclerosis, young adult males vs. females, Hispanics vs. whites, and children from economically less-developed vs. more developed regions. Cases of nodular lymphocyte predominance HD\textsuperscript{30} virtually never contain EBV. Geographic, cultural, genetic, or socioeconomic influences seem to be important. Whereas 30–50% of cases of HD from the United States, most parts of Europe, and Israel have been shown to contain EBV-positive Reed-Sternberg cells, in Central and South America and in Africa the prevalence has been much higher. The incidence of EBV positivity in Asian populations may also be higher than that of Western populations. In England, low socioeconomic status is linked to an increased incidence of EBV in childhood HD.\textsuperscript{26}

Risk factors for EBV-positive Hodgkin’s disease are almost the inverse of risk factors for young adult HD. Among young adult cases, where the poliovirus hypothesis is most relevant, EBV positivity in tumor tissue is generally lowest.\textsuperscript{25} The association with EBV is highest in underdeveloped countries, children, and the aged. Thus the polio model as applied to delayed EBV infection and HD is either wrong or grossly oversimplified.

EBV positivity as a prognostic variable remains of uncertain importance, with some studies indicating that the presence of the virus is a positive prognostic factor, while other unpublished studies suggest it is a negative prognostic factor in the very young and the very old.\textsuperscript{27,28}

How tumor cells expressing viral antigens elude immune surveillance in Hodgkin’s disease is not yet clear. However, several possible explanations have been investigated. The MHC class I antigen presentation machinery is present in EBV(+) HD. Although in EBV(-) HD MHC class I is characteristically not expressed, in EBV (+)HD it is expressed.\textsuperscript{23,29} Furthermore, other elements of the antigen processing machinery are present and appear to be functional. Mutation or variation in expressed viral protein epitopes is minimal and seems inadequate to explain resistance to tumor killing. Evidence has been presented that class II antigen presentation is not functional.\textsuperscript{30} Local suppression of EBV immunity (in the vi-
Viral hemorrhagic fevers (VHFs) are viral illnesses characterized by fever, hemorrhage, and multisystem organ dysfunction, often leading to deaths in epidemic settings. With increasing international travel, the potential danger of transmission and importation of non-endemic VHFs has been the focus of intense media attention and public concern. VHFs syndromes associated with human disease are caused by RNA viruses divided into families: Arenaviridae, Bunyaviridae, Filoviridae and Flaviviridae. Each of the four distinct families has specific geographic patterns with vector and animal reservoirs (usually asymptomatic viremic carriers) (Table 1). Although the individual disease pattern may differ for each virus, the hemorrhagic viruses share many common features (Table 2).

### Clinical Manifestations of Hemorrhagic Fever

**Viruses: An Overview**

The clinical syndrome of a VHF usually begins with fever, myalgia, and malaise and then progresses to dramatic multiorgan system failure. Vascular dysregulation with hypotension, flushing, and injected conjunctivae precede or become concomitant with capillary leak, hemorrhage and shock. Hemorrhage into organs, effusions in serous cavities and widespread necrosis can occur in any organ system. Liver and lymphoid systems are almost always involved, and the lung has varying degrees of interstitial pneumonitis and hemorrhage. Inflammatory response or neutrophil infiltration is never striking. Lack of inflammatory response has been attributed to immune suppression by viral glycoprotein production and/or cytokine production.

### Mechanism of Hemorrhage in Viral Hemorrhagic Fevers

The pathogenesis of hemorrhage in patients with VHFs has been unclear until very recent work with the Ebola virus. Clearly, thrombocytopenia, platelet dysfunction and disseminated intravascular coagulation (DIC) have been noted in VHFs but never to the degree consistent with the overwhelming hemorrhage. Both decreased marrow production and increased platelet consumption have been implicated in the pathogenesis of VHF thrombocytopenia. Mild coagulation abnormalities have been reported, usually in the setting of liver dysfunction. When DIC occurs, it is usually mild and does not play a significant role in bleeding. For example, in patients and monkeys infected with Lassa virus, platelet counts usually remain above 100,000/ml and PT, PTT, fibrinogen and FSPs remain relatively normal.

A platelet inhibitor isolated from plasma of severe Lassa cases that demonstrates marked depression of platelet aggregation. Plasma from patients infected with Junin virus (Argentine hemorrhagic virus) also demonstrated a platelet inhibitor. Evidence for the presence of immune complexes on platelet surfaces has been reported in two of the viral hemorrhagic fevers, dengue HF and hemorrhagic fever renal syndrome (HFRS). Platelet kinetic studies have established increased platelet consumption as a cause of thrombocytopenia in dengue and HFRS.

The most provocative explanation for the severe hemorrhage seen in VHFs has come from work with endothelial cell injury. Recent work has demonstrated that Ebola virus infects endothelial cells lining blood vessels and destroys these vessels. A viral glycoprotein (GP) has been identified that is responsible for infecting endothelial cells and causing vascular cell cytotoxicity and injury. Gene transfer of this GP into explanted human or porcine blood vessels caused massive endothelial loss and vascular leak within 48 hours. Modification of a part of this GP has recently been achieved that permitted viral infection of endothelial cells without cytotoxicity lending hope for a molecular target for potential vaccines or antivirals. Table 3 reviews mechanisms of hemorrhage in the VHFs.

### Clinical Manifestations of Specific VHFs Causing Disease in Humans

**Flaviviridae: Dengue**

There are four dengue serotypes: DEN-1, DEN-2, DEN-3, and DEN-4. They belong to the genus Flavivirus, family Flavividae. Infection with one dengue serotype provides cross-protective immunity to that serotype and no cross-protective immunity to other serotypes. Humans are infected by *Aedes aegypti* mosquito, a highly domesticated urban mosquito commonly found around homes.
### Table 1. Epidemiology of viral hemorrhagic fevers (VHFs).

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Virus</th>
<th>Human Disease</th>
<th>Transmission Routes</th>
<th>Reservoir</th>
<th>Incubation Period</th>
<th>Endemic Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arenaviridae</td>
<td>Arenavirus</td>
<td>Guanarito</td>
<td>Venezuelan HF</td>
<td>Rodent to human, via direct contact and by aerosolization of body fluids.</td>
<td>Wild rodent</td>
<td>7-16 days</td>
<td>Venezuela</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Junin</td>
<td>Argentine HF</td>
<td>Same as above</td>
<td>Wild rodent</td>
<td>7-16 days</td>
<td></td>
<td>Argentina</td>
</tr>
<tr>
<td></td>
<td>Machupo</td>
<td>Bolivian HF</td>
<td>Same as above</td>
<td>Wild rodent Calomys callosus</td>
<td>7-16 days</td>
<td></td>
<td>Northern Bolivia</td>
</tr>
<tr>
<td></td>
<td>Sabiá</td>
<td>Brazilian HF</td>
<td>Same as above, laboratory acquired.</td>
<td>?Rodent</td>
<td>7-16 days</td>
<td></td>
<td>Brazil</td>
</tr>
<tr>
<td></td>
<td>Lassa</td>
<td>Lassa fever</td>
<td>Same as above</td>
<td>Mastomys natalensis rodent</td>
<td>5-21 days</td>
<td></td>
<td>West Africa</td>
</tr>
<tr>
<td>Bunyaviridae</td>
<td>Phlebovirus</td>
<td>Rift Valley Fever</td>
<td>Rift Valley fever</td>
<td>Mosquito-borne, contact with livestock, nosocomial spread</td>
<td>Wild and domestic</td>
<td>2-5 days</td>
<td>Sub-Saharan Africa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mammals</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nairovirus</td>
<td>Crimean Congo HF</td>
<td>Crimean Congo HF</td>
<td>Hyalomma tick borne, contact with infected animals, nosocomial spread.</td>
<td>Hares, domestic animals, birds, ticks</td>
<td>3-12 days</td>
<td>East Europe, Africa, Middle East, China</td>
</tr>
<tr>
<td></td>
<td>Hantavirus</td>
<td>Hantaan</td>
<td>Hemorrhagic fever with renal syndrome (HFRS)</td>
<td>Rodent to human. Direct contact vs. aerosolization of rodent body fluids</td>
<td>Wild rodents</td>
<td>9-35 days</td>
<td>Worldwide, especially in Asia (endemic China), Europe</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Puumala</td>
<td>HFRS</td>
<td>See above</td>
<td>See above</td>
<td>9-35 days</td>
<td>Europe</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seoul</td>
<td>HFRS</td>
<td>See above.</td>
<td>See above</td>
<td>9-35 days</td>
<td>?Worldwide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sin Nombre, New York-1, Bayou, Rio Mamore, Laguna Negra and others</td>
<td>Hantavirus pulmonary syndrome</td>
<td>See above.</td>
<td>See above</td>
<td>7-28 days</td>
<td>Americas</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>Filovirus</td>
<td>Ebola</td>
<td>Ebola HF</td>
<td>Unknown</td>
<td>Person to person Monkey to human, Nosocomial</td>
<td>3-16 days</td>
<td>Western Sub-Saharan Africa, Philippines</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Marburg</td>
<td>Marburg HF</td>
<td>Unknown</td>
<td>See above</td>
<td>3-16 days</td>
<td>Western Sub-Saharan Africa</td>
</tr>
<tr>
<td>Flaviviridae</td>
<td>Dengue (types 1-4)</td>
<td>Dengue HF</td>
<td>Aedes aegyti and Ae. albopictus mosquito-borne</td>
<td>Monkeys, Humans</td>
<td>3-15 days</td>
<td></td>
<td>Worldwide, Hemorrhagic syndromes occur in Southeast Asia and Caribbean.</td>
</tr>
<tr>
<td></td>
<td>Kyasanur Forest Disease</td>
<td>Kyasanur Forest Disease</td>
<td>Ixodid tick-borne</td>
<td>Monkeys, rodents, shrews</td>
<td>3-8 days</td>
<td></td>
<td>Kamataka State, India, Pakistan</td>
</tr>
<tr>
<td></td>
<td>Omsk</td>
<td>Omsk Hemorrhagic Fever</td>
<td>Ixodid tick-borne, muskrats</td>
<td>Muskrat to human</td>
<td>3-8 days</td>
<td></td>
<td>Siberia</td>
</tr>
<tr>
<td></td>
<td>Yellow Fever</td>
<td>Yellow Fever</td>
<td>Mosquito-borne (Aedes aegyti), Amblyomma ticks (rare)</td>
<td>Monkeys, Humans</td>
<td>3-6 days</td>
<td></td>
<td>South American, West and East Africa</td>
</tr>
</tbody>
</table>

*Hematology 2000*
Most hemorrhagic viruses are transmitted by infectious agents that are arthropod-borne (mosquitoes, ticks); however, person-to-person transmission may occur with five viruses through direct contact with blood or secretions: Lassa, Marburg, Congo-Crimean, Ebola, Sabiá. Animal excreta have also been implicated in transmission.

Asymptomatic animal reservoirs of viruses are generally rodents (Hantavirus, Lassa, Guanarito, Junin, Machupo) although monkeys and primates may perpetuate disease through a sylvatic cycle (yellow fever, dengue). Many viruses have not had reservoirs identified (Ebola, Marburg, Sabiá).

Clinical manifestations of VHF are associated with a short incubation period (usually less than 21 days). Clinical pathology has common themes: capillary leak, thrombocytopenia, leukopenia, DIC and hepatocellular destruction are often described. Early vascular dysregulation with hypotension, flushing, and injected conjunctivae is commonly followed by hemorrhage, shock and multi-organ dysfunction. Milder undifferentiated febrile illnesses and subclinical infections with asymptomatic seroconversion and potential immunity have also been described for all of the VHF viruses.

Diagnosis of VHF depends upon the demonstration of the infecting virus in an acute serum sample by antigen-detection ELISA or reverse transcription and subsequent polymerase chain reaction (RT-PCR). IgM antibodies can also be helpful in making a diagnosis in early convalescence by IgM capture ELISA technique. Classical histopathology in autopsy specimens may unfortunately be the first clue.

Supportive therapy is the mainstay of management of most VHF's, better accomplished in countries with sophisticated technological support that is often lacking in endemic developing countries. Antiviral therapy with ribavirin is recommended for all Arenaviridae and Bunyaviridae infections (with the exception of Hantavirus pulmonary syndrome). Unfortunately, ribavirin is often difficult and expensive to obtain in an endemic setting.

Control measures generally involve meticulous hospital infection control efforts, strict isolation for certain agents, concurrent disinfection, and contact and source reporting to public health authorities. Vaccines can protect against some of the VHF viruses.

Table 2. Shared features of hemorrhagic viruses.

- Most hemorrhagic viruses are transmitted by infectious agents that are arthropod-borne (mosquitoes, ticks); however, person-to-person transmission may occur with five viruses through direct contact with blood or secretions: Lassa, Marburg, Congo-Crimean, Ebola, Sabiá. Animal excreta have also been implicated in transmission.
- Asymptomatic animal reservoirs of viruses are generally rodents (Hantavirus, Lassa, Guanarito, Junin, Machupo) although monkeys and primates may perpetuate disease through a sylvatic cycle (yellow fever, dengue). Many viruses have not had reservoirs identified (Ebola, Marburg, Sabiá).
- Clinical manifestations of VHF are associated with a short incubation period (usually less than 21 days). Clinical pathology has common themes: capillary leak, thrombocytopenia, leukopenia, DIC and hepatocellular destruction are often described. Early vascular dysregulation with hypotension, flushing, and injected conjunctivae is commonly followed by hemorrhage, shock and multi-organ dysfunction. Milder undifferentiated febrile illnesses and subclinical infections with asymptomatic seroconversion and potential immunity have also been described for all of the VHF viruses.

Clinical features

Dengue virus infection in humans causes a spectrum of illness, ranging from inapparent or mild febrile illness to severe and fatal hemorrhagic disease. After a person is bitten by an infective mosquito, asymptomatic viremia occurs (average, 4 to 7 days), after which the person may experience acute onset of fever accompanied by a variety of nonspecific signs and symptoms. Infection with all four serotypes causes a similar clinical presentation that may vary in frequency and severity. Classic dengue fever, “break-bone fever,” is primarily a disease of older children and adults. It is characterized by sudden onset of fever and a variety of nonspecific signs and symp-

Table 3. Mechanisms of hemorrhage in viral hemorrhagic fevers (VHFs).

<table>
<thead>
<tr>
<th>Disease</th>
<th>Thrombocytopenia</th>
<th>Platelet Dysfunction</th>
<th>Reduced Levels of Coagulation Factors</th>
<th>Disseminated Intravascular Coagulation</th>
<th>Vascular Injury (Endothelial Cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentine HF</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bolivian HF</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lassa fever</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dengue HF</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow fever</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omsk HF</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyasanur Forest disease</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crimean-Congo HF</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rift Valley fever</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF with renal syndrome</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ebola HF</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marburg HF</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: + = demonstrated abnormality or data compatible with an abnormality; … = data insufficient; ± = data equivocal

A Occurs in terminal shock.
B Platelet inhibitor discovered in sera of severely ill patients
C Diagnosis complicated by presence of severe liver dysfunction
D Monkeys

Modified from 14

in the tropics. The mosquitoes are day feeders and often infect several members in the same household.

Clinical features

Dengue virus infection in humans causes a spectrum of illness, ranging from inapparent or mild febrile illness to severe and fatal hemorrhagic disease. After a person is bitten by an infective mosquito, asymptomatic viremia occurs (average, 4 to 7 days), after which the person may experience acute onset of fever accompanied by a variety of nonspecific signs and symptoms. Infection with all four serotypes causes a similar clinical presentation that may vary in frequency and severity. Classic dengue fever, “break-bone fever,” is primarily a disease of older children and adults. It is characterized by sudden onset of fever and a variety of nonspecific signs and symp-
toms, including frontal headache, retro-orbital pain, body aches, nausea and vomiting, joint pains, weakness, and rash. The temperature may initially rise to 102–105°F and last for 2 to 7 days. The conjunctivae may be injected and the pharynx inflamed. Facial flushing or erythematous mottling may occur coincident with or slightly before onset of fever and disappear 1 to 2 days after onset of symptoms. A second rash beginning on the trunk may appear between days 2 and 6 of illness and spread to the face and extremities. Toward the end of the febrile illness or after the temperature reverts to normal, petechiae may appear; these may be scattered or confluent. Intense pruritus followed by desquamation on the palms of the hands and soles of the feet may occur.

Dengue hemorrhagic fever (DHF) is primarily a disease of children under the age of 15 years, although it may also occur in adults. Prior immunity to a serotype of dengue infection is a feature of more than 90% of cases, resulting in immune enhancement and massive release of cytokines with vasoactive properties. These vasoactive mediators cause capillary leakage, circulatory failure and disseminated intravascular coagulopathy. Skin hemorrhages are the most common, including petechiae and purpura, as well as gum bleeding, epistaxis, menorrhagia, and gastrointestinal hemorrhage. Blood tests will usually show that the patient has thrombocytopenia (platelet count of less than 100,000/mL) and evidence of a vascular leak syndrome (hemoconcentration with a hematocrit rise of > 20%). The tourniquet test, done by inflating the blood pressure cuff to the midpoint between the systolic and diastolic pressures for 5 minutes and then releasing the pressure, will “shower” petechiae below the cuffline in persons with increased capillary fragility.

Clinical laboratory findings associated with dengue include neutropenia followed by lymphocytosis, often marked by atypical lymphocytes. Liver enzymes in the serum may be mildly elevated, and in some patients alanine transaminase and aspartate transaminase levels may reach 500 to 1000 units/L. Thrombocytopenia is also common in dengue fever; in one epidemic, 34% of confirmed dengue fever patients who were tested had platelet counts of less than 100,000/mL. Dengue fever is generally self-limited and rarely fatal. The acute phase of illness lasts for 3 to 7 days, but the convalescent phase may be prolonged for weeks and may be associated with weakness and depression. Unfortunately, DHF has a high mortality rate from complications of hemorrhage and shock.

Diagnosis

Virus can often be isolated from acute phase blood samples taken in the first 5 days of illness. Viral RNA can often be detected by polymerase chain reaction (PCR) in serum. The IgM capture enzyme-linked immuno-sorbent assay (ELISA) detects IgM antibody, which usually appears by day 5 after onset and persists for 2 to 3 months. The hemagglutination-inhibition test and an IgG ELISA detect IgG antibody, which appears simultaneously or shortly after IgM and persists for life.

Prevention

Although there is progress in developing a vaccine against dengue viruses, no vaccine is currently available. The most effective way to control the mosquitoes that transmit dengue is larval source reduction. Personal protection can be achieved by using DEET compounds on skin, sleeping under permethrin-impregnated netting and/or spraying clothing with permethrin compounds.

Flaviviridae: Yellow fever

Epidemiology

Yellow fever virus is a member of Flaviviridae genus (flavi means “yellow”) and is found in the tropical Americas and sub-Saharan Africa. Official reports from endemic areas involve about 3000 cases per year, but recent epidemics in Nigeria, Kenya and the Amazon area have raised the annual incidence and signalled a re-emergence of disease. Urbanization of populations near forested areas has recently caused urban yellow fever, a cycle involving only mosquitoes and infected humans.

Clinical features

Yellow fever carries a case-fatality rate of about 20%. It exists in two transmission cycles: a sylvatic or jungle cycle that involves mosquitoes and infected non-human primates, mostly monkeys, and an urban cycle, involving mosquitoes and infected humans. *Ae. aegypti* is the usual mosquito vector, but several other species have been implicated, including *Amblyomma* ticks.

After a person is bitten by a mosquito infected with yellow fever virus, the usual incubation period is 3–6 days. Most infected individuals suffer only mild illness with fever and malaise. Serious illness is manifested by three phases. The acute phase is characterized by fever, headache, myalgia, nausea and vomiting; few physical specific signs are apparent at this stage except for Faget’s sign, a relative bradycardia with fever. The second phase is a period of “remission,” when the fever remits for 1–2 days. During the third phase, the “period of intoxication,” fever recurs accompanied by jaundice and hemorrhagic manifestations. “Black vomit” refers to the massive hematemesis that can occur; the high mortality of this phase can often be related to the liver failure, myocarditis, encephalopathy and acute renal failure that occurs during this phase.
Laboratory findings include leukopenia, thrombocytopenia and abnormal coagulation parameters. Leukocytosis can evolve, as can elevated transaminases, hyperbilirubinemia and hypoglycemia, all indicating incipient liver failure. Nephrotic range proteinuria and renal failure can occur. Yellow fever infection can be detected early in serum or blood by using IgM-antibody capture ELISA or PCR; IgM-specific ELISA appears by the end of the first week. A 4-fold or greater rise in titer in serum plaque-neutralizing antibody, complement fixation, or hemagglutination-inhibition antibodies is also diagnostic but requires paired acute and convalescent sera. Cell culture can detect virus in acute serum. Pathologic examination of liver with viral isolation provides a postmortem diagnosis, as antemortem liver biopsy is usually contraindicated because of risk of bleeding.

Treatment and prevention
No effective antiviral agent is available. All treatment is supportive. Index patients should be protected from mosquito bites for 5 days after illness to avoid spread. Blood and needle precautions should be instituted by health care attendants. The best preventative measure against yellow fever infection is the live attenuated 17D vaccine. A single subcutaneous injection is immunogenic in 99% of recipients and probably offers lifelong immunity, although 10-year boosting is suggested if travel to an endemic area is anticipated.

Arenaviridae\textsuperscript{3,5-7}: Lassa

\textbf{Epidemiology}
Lassa fever virus is a member of the Arenaviridae genus and is endemic as well as epidemic in West Africa. It is estimated that there are 400,000 hospital admissions yearly for Lassa fever in West Africa, with 5,000 of these admissions resulting in death.\textsuperscript{5}

\textbf{Clinical features}\textsuperscript{5}
The vector for Lassa fever is the bush rodent \textit{Mastomys natalensis}. Humans are infected when they come into contact with excreta or aerosolized body fluids from infected mice, via contact with an infected human, or from nosocomial transmission. Human-to-human infection is unlikely to be caused by inhalation of respiratory droplets, but rather from direct contact with human bodily fluid.

After an incubation period of 5–21 days, the acute phase of Lassa fever usually lasts between one and four weeks. In contrast to other hemorrhagic fevers, its onset is characteristically insidious, featuring fever, severe sore throat and headache, back pain and abdominal pain. The late phase of disease involves its hemorrhagic manifestations. In particular, the hemorrhagic manifestations are gastrointestinal, with death occurring from hypovolemic shock secondary to hemorrhage. Other late-stage features are relative bradycardia, pleural/pericardial effusions, pneumonitis, encephalopathy, facial edema, and endothelial and platelet dysfunction. DIC, however, does not occur.\textsuperscript{6}

The mortality rate of clinically apparent Lassa fever is estimated at 15%. A mortality rate of 80% is associated with an elevated AST (> 150 IU/L) and high levels of viremia. (> 10^{14} \text{TCID}_{50} \text{ per ml}). Also, there is an increased mortality rate with pregnancy.

\textbf{Diagnosis}
Clinical suspicion is paramount for diagnosing Lassa fever in patients who present in an epidemic setting with the clinical triad of pharyngitis, retrosternal pain and proteinuria.\textsuperscript{3} Utilizing acute serum via the ELISA test IgG or IgM, antibodies can be detected. Second, detection of a 4-fold or greater rise in IgG serum titer, using paired acute and convalescent titers, can be diagnostic.\textsuperscript{5} Viral antigen can be detected by reverse transcriptase PCR (RT-PCR) of acute serum. Finally, diagnosis by isolation of virus in cell culture is possible if the patient is acutely ill. Autopsy findings display focal necrosis of the spleen, liver and adrenals.\textsuperscript{3}

\textbf{Treatment}\textsuperscript{7}
Patients treated with intravenous or oral ribavirin within the first 6 days of fever have a statistically significant decrease in mortality rate; ribavirin is also more effective than administering convalescent plasma. Ribavirin is recommended for all phases of the disease, including post-exposure prophylaxis.\textsuperscript{7} If it is available, intravenous ribavirin should be chosen over oral ribavirin for severely ill patients. Prophylactic oral ribavirin is recommended for all non-pregnant close contacts.\textsuperscript{7}

\textbf{Prevention}
Prevention includes strict isolation and barrier precautions with prompt notification of local and national public health authorities.\textsuperscript{2} Control of rodents and proper storage of food may control rodent-to-human transmission. Prophylaxis with oral ribavirin of close or high-risk contacts should be considered (see Table 4 for definition of high-risk and close contacts). There is no commercially available vaccine.

Filoviridae\textsuperscript{3,10,11}: Ebola and Marburg Viruses

Ebola and Marburg viruses are the two members of the Filoviridae genus. Both are enveloped, single-stranded RNA viruses. These two viruses can be genetically, serologically and biochemically distinguished.\textsuperscript{3}
Table 4. Contacts and surveillance.

- **Casual contact:**
  Person who had remote contact with ill patient (such as same plane, same hotel).
  **Surveillance:**
  No special surveillance needed.

- **Close contact:**
  Household contact, patient care providers, laboratory handlers, shaking hands, hugging.
  **Surveillance:**
  Temperature taken twice daily for 3 weeks with immediate reporting of fever or symptom to surveillance team.

- **High-risk contact:**
  Mucous membrane contact (kissing, intercourse, penetrating injury with patient's excretions, secretions or blood).
  **Surveillance:**
  Twice daily temperature as above.
  Isolation with fever and treat as VHF until diagnosis excluded by culture.
  Consider post-exposure ribavirin prophylaxis, if sensitive virus such as any Arenaviridae or Bunyaviridae (except Hantavirus pulmonary syndrome).

Marburg epidemiology

Marburg virus was first described in 1967, in commercial laboratory workers in Marburg, Germany presenting with VHFIs. Infected green monkeys imported from Uganda for research purposes were the identified carriers. Isolated cases have subsequently been reported in South Africa, Zimbabwe, and Kenya. No reservoir has been identified.

Ebola epidemiology

Ebola virus is a member of the Filoviridae genus and has been described in Western Sub-Saharan Africa and from imported monkeys from the Philippines. Ebola was first described in 1976, when two outbreaks coincided in southern Sudan and northern Zaire. Ebola is the name of a small river in northwestern Zaire. Ebola is subdivided into four genetically distinct subtypes: Zaire (EBO-Z), Sudan (EBO-S), Reston (EBO-R), and more recently Ivory Coast (EBO-CI).

Characteristics of Marburg and Ebola viruses

For both Marburg and Ebola viruses, the natural reservoir is unknown, although zoonotic transmission is postulated. The original Marburg outbreak in Germany was associated with ill green monkeys (Cercopithecus aethiops) imported from Uganda. Likewise, the Reston, Virginia, Ebola outbreak was linked to infected symptomatic cynomolgus monkeys (Macaca fascicularis) imported from the Philippines, but as in all other cases, no uninfected animal reservoir was identified.

Human-to-human transmission via intimate contact is documented in each disease. Nosocomial transmission is clearly documented via infected body fluids. Generally, transmission has occurred between close contacts by sexual transmission and from contaminated needles and syringes: not by casual contact.

Clinical features

The incubation period for Marburg and Ebola viruses is estimated at 3–16 days. Marburg virus carries a mortality rate of 23%, while Ebola virus has a wider range of mortality rate of 53–88%, varying by Ebola strain and available health care resources. The clinical features of Marburg and Ebola hemorrhagic fevers exhibit a biphasic illness pattern, punctuated by a remission-like period. The initial phase is characterized by asthenia/weakness, the later phase by hemorrhage. The first week of illness (the “initial phase”) is characterized by the acute onset of fever, headache, myalgias, and arthralgias. Next, patients experience conjunctivitis, vomiting, and non-bloody diarrhea with abdominal pain. A maculopapular rash with resultant desquamation occurs in approximately one-half of patients, usually by the fifth day of illness. Additionally, on the fifth day of illness, patients start to develop signs of petechiae and hemorrhage.

Patients with Marburg virus tend to develop ananthesia with mucosal erythema and eruption. Ebola cases show no ananthesia, but patients complain of an intensely dry, sore throat with odynophagia and also may complain of chest pain. Severely ill Ebola patients often exhibit profound lethargy and prostration, with deep-set eyes and expressionless faces, described as “ghost-like.”

The “remission” phase of filovirus infection lasts for 24–48 hours before the second phase of morbidity. The “late phase” of disease, during the second week of illness, exhibits normothermia, tachypnea, shock, oliguria, and hemorrhage. Hepatitis with elevated transaminases occurs in both diseases. In Ebola patients, encephalopathy is common. Hemorrhage has been reported in 45–78% of cases.

Death, usually occurring in the second week of illness, has been associated with mucosal bleeding, oozing from puncture sites, anuria, hiccups, tachypnea and encephalopathy. Convalescence is often prolonged, lasting weeks, and can include orchitis, ocular diseases, arthralgias and alopecia. Laboratory features include thrombocytopenia and early lymphopenia followed by a neutrophilic leukemoid response. Transaminitis (AST greater than ALT), icterus, markedly elevated LDH, and increased amylase are all prominent. Urinalysis reveals hematuria and proteinuria.
Diagnosis\textsuperscript{3,4,10,11}

There are several methods for detecting the Marburg and Ebola viruses in the laboratory. Both can be isolated from the serum by electron microscopy. IgM-antibody capture ELISA tests are usually positive early in convalescence and can be coupled with rising IgG antibody titers. RT-PCR can be used to detect low concentrations of Ebola viral RNA. At autopsy, Marburg and Ebola viruses can be isolated from serum, blood, urine, pharyngeal swabs, semen, liver, and lymphoid tissue. Virus is detectable in semen for up to three months after recovery from infection, and thus may be sexually transmissible.

Treatment

As there is no known therapy for either viral hemorrhagic fever, supportive care is offered. Several trials have shown there is no efficacy for the use of interferon-alpha, convalescent plasma, or ribavirin.

Prevention\textsuperscript{1,2}

If Ebola or Marburg hemorrhagic fever is suspected, strict isolation of the index case is essential. Barrier precautions with gowns, gloves and masks are required. Biosafety level 4 precautions are to be implemented, and local and national health authorities should be contacted immediately. All non-human primates suspected of disease should be quarantined.

Bunyaviridae\textsuperscript{8,9}: Crimean Congo Hemorrhagic Fever

Epidemiology\textsuperscript{3}

Crimean Congo hemorrhagic fever (CCHF) is caused by the single-stranded RNA \textit{Nairovirus} of the Bunyaviridae genus. CCHF has been identified in Africa, the Middle East, the Balkans, Russia and Western China. It was first recognized in 1944 in Crimea as a hemorrhagic febrile illness.

The vectors are the ixodid ticks in the \textit{Hyalomma} genus. Transmission is thought to occur between humans and from the blood, aerosols or fomites from slaughtered cattle and sheep. Tick bites and direct handling or crushing of ticks is another route of transmission. Nosocomial infections via blood have been well documented. Community epidemics in endemic areas are postulated to occur via aerosol spread. Subclinical or asymptomatic infections are thought to occur in endemic areas.

Clinical features\textsuperscript{3}

CCHF is generally an acute and self-limiting disease, except in the event of an epidemic. Onset of disease is acute. After an incubation period generally of 3-6 days (but up to 12 days, especially in nosocomial cases), patients experience fever, severe headache, chills, arthralgias, vomiting and prostration. Myalgias are concentrated in the lower back and limb area. The soft palate often develops a fine petechial rash, and the pharynx can be hyperemic.

Clinically, CCHF has the most severe bleeding and ecchymoses of all the hemorrhagic fevers. On day four or five of illness, severe hemorrhage occurs in up to 25% of cases. Hemorrhagic manifestations include gum bleeding and epistaxis, followed by gastrointestinal hemorrhage. Ecchymoses are often large and pressure-linked. Hematuria, proteinuria, azotemia, and liver involvement are associated with poor prognosis. Mortality can be from 15 to 70%.

Diagnosis\textsuperscript{3}

Laboratory values feature thrombocytopenia, hemorrhage, leukopenia, and icterus. Virus or antibodies should be detectable within 7–20 days after the onset of clinical symptoms. Additionally, virus isolation from the serum of severely ill cases is usually positive in cell culture. Antibodies can be detected by complement fixation, hemagglutination-inhibition, ELISA, neutralization and immunofluorescence techniques. Acute and convalescent serum can be used to identify virus-specific antibodies. RT-PCR can be performed on sera of acutely ill patients.

Treatment\textsuperscript{3}

Therapy for CCHF consists of rapid clinical diagnosis followed by the implementation of supportive therapy. Convalescent immune plasma therapy is not associated with improved outcome in patients with CCHF. In vitro, Crimean Congo virus is susceptible to ribavirin. Extrapolating from studies of the treatment of Lassa fever with ribavirin, intravenous ribavirin is the preferred route of administration.

Prevention\textsuperscript{3}

Prevention of CCHF entails the avoidance of exposure to tick bites. The slaughter of acutely viremic livestock may increase the risk of disease transmission. In appropriate areas, cattle dipping (to reduce the number of infected vectors) may decrease transmission rates to humans. Barrier nursing is essential in the clinical setting. A formalin-inactivated vaccine has been used regionally in high-risk groups but is of limited efficacy (C.J. Peters, personal communication).

Approach to a Suspected Case of VHF in a Community Setting

When travel, such as exposure to an endemic area within 3 weeks, or occupational history combine with physical signs suggesting a VHF, such as fever, pharyngitis, conjunctivitis, skin rash that predate rapid hemorrhage and shock, the following four actions should immediately be taken:
1. Isolate the patient with barrier nursing precautions (see Table 5).
2. Notify local, state health department and CDC—phone: 404-639-1115; 404-639-1511 or 404-639-2888 (after hours and weekends).
3. Determine the biosafety level to be achieved for the virus suspected.
4. Begin surveillance of all contacts of index case since illness (Table 4).

Collecting and Handling Laboratory Specimens
Ideally, all hospital workers drawing blood or collecting secretions should ideally be responsible for double-bagging specimens, disinfecting exterior bagging with bleach and hand carrying specimens to the laboratory. Laboratory specimens should be disinfected with TritonX, a detergent, prior to automated machine use.

Treatment Regimens
Ribavirin has been used with some success for most Bunyaviridae and Arenaviridae infections. Intravenous treatment can result in a mild hemolysis and transient LFT abnormalities. Ideally all VHF patients should be aggressively supported in an intensive care setting.

Contact Surveillance
Identification and surveillance of all patient contacts is essential to disease control. A contact is defined as a person who has been exposed to the secretions, excretions or tissues of an index case within 3 weeks of illness. Contacts may be divided into 3 levels of risk. Surveillance methods are described in Table 4. A team should be identified to cover a telephone hot-line permitting 24-hour surveillance of contacts. Although increasing international travel increases the potential danger of an imported VHF, a good epidemiologic history coupled with heightened vigilance for clinical signs can permit early diagnosis and safe management of an index VHF case presenting in a community setting.

Table 5. Isolation management of a potential VHF carrier.

| Isolate using universal precautions. |
| A negative pressure room should be arranged, preferably with an anteroom for removing protective barriers and storing supplies. |
| All nonessential staff and visitors should be restricted. |
| Gloves and gowns should be worn by all persons entering room. |
| Face shields, surgical masks, and eye protection should be worn by persons coming within 3 feet of patient. |
| If a suspected VHF case has a prominent cough, vomiting, diarrhea or hemorrhage, personal protective respirators are recommended (high efficiency particulate air respirators). |
| Chemical toilets should be used and all effluents disinfected with bleach prior to disposal into a municipal sewer system for up to 6 weeks of convalescence or until patient is virologically negative. |
| Soiled linens should be double-bagged and either incinerated or autoclaved. Hot water cycle with bleach can be used if no sorting occurs. |

Modified from 1,2

REFERENCES
I. HTLV Types I and II
16. Nicot C, Astier-Gin T, Guillemain B. Activation of Bcl-2 expression in human endothelial cells chronically expressing the
II. Epstein-Barr Virus and Hodgkin’s Disease


III. Viral Hemorrhagic Fevers

General

Arenaviridae

Bunyaviridae

Filloviridae

Flaviviridae

Hemorrhagic manifestations of VHFs