epithelial and alveolar cells harvested from experimentally infected mice also exhibit apoptotic changes, suggesting that this mechanism of cell death may be important in the pathogenesis of influenza in vivo.[163] The specific mechanism by which influenza virus induces apoptosis is unclear, but it may be related to induction of Fas antigen by double-stranded RNA during virus replication.[164] An unusual viral protein of influenza A viruses, encoded by a second open reading frame in the *PB1* gene and therefore referred to as PB1-F2, also plays a role in induction of apoptosis by poisoning mitochondria.[19]

Virus release continues for several hours before cell death ensues. Released virus then may initiate infection in adjacent and nearby cells, so within a few replication cycles a large number of cells in the respiratory tract are releasing virus and dying as a result of the virus replication. The time between the incubation period and the onset of illness and virus shedding varies from 18 to 72 hours depending in part on the inoculum dose.[165]

Influenza virus infection of peripheral blood mononuclear cells (PBMCs), including polymorphonuclear leukocytes (PMNs), lymphocytes, and monocytes, is nonproductive, but it is associated with measurable defects in cellular function that may be relevant to the pathogenesis of influenza-related infectious complications. These include defects in PMN chemotaxis and phagocytosis,[166] and decreased proliferation and costimulation by mononuclear cells.[167] The effects are mediated by virus replication and possibly by a direct toxic effect of certain virus proteins, including hemagglutinin, neuraminidase, and nucleoprotein.[168] It has been noted that the short portion of the sequence of the influenza A virus NP is homologous to a naturally occurring peptide found in normal bronchoalveolar lavage fluid that inhibits PMN chemotaxis and oxidative burst,[169] and that exposure of PMNs to influenza virus suppressed endocytosis by these cells.[170]

Virus Shedding

Quantitation of virus in respiratory tract specimens reveals a characteristic pattern (Fig. 165.7). Virus is first detected just before the onset of illness (within 24 hours), rapidly rises to a peak of 3.0 to 7.0 $\log_{10}$ tissue-culture infective dose [$TCID_{50}$]/mL, remains elevated for 24 to 48 hours, and then rapidly decreases to low titers.[171] Usually, virus is no longer detectable after 5 to 10 days of virus shedding. However, because of the relative lack of immunity in the young, more prolonged shedding of higher titers of virus is seen in children. Children also appear to exhibit heterogeneous shedding, with the top 20% of shedders estimated to represent 90% of infectiousness.[172]

The severity of illness correlates temporally with quantities of virus shed in experimental influenza in volunteers, thus suggesting that a major mechanism in the production of illness is cell death resulting from viral replication. Although the clinical manifestations of influenza are dominated by systemic symptoms, viral replication is limited to the respiratory tract. However, virus can be detected with PCR in peripheral blood in immunocompromised patients and may be a marker for more severe outcomes.[173]

Cytokine Response

Systemic symptoms have been linked to the release of potent cytokines, such as type I interferons (IFNs), tumor necrosis factor, and interleukins (ILs), by infected cells and responding lymphocytes.[171] In fact, it has been suggested that an overly vigorous cytokine response to infection may contribute to the high fatality rate seen with H5N1 influenza,[174,175] sometimes referred to as a cytokine storm.

Studies have suggested that defects in the cytokine response may increase the risk of severe influenza. Single nucleotide polymorphisms (SNPs) in the gene for IFN-inducible transmembrane 3 (IFITM-3) have been associated with cytokine dysfunction and increased severity of influenza.[176] Similar SNPs have also been identified as associated with increased severity of human H7N9.[177] Carriers of the risk allele were shown to have decreased levels of $CD8^+$ T cells in their airways during influenza infection, suggesting that a critical function of this protein may be to promote immune cell persistence at mucosal sites.[178] IFN deficiency related to mutations in IRF7 have also been contributory to severe influenza in a human child.[179] Mutations in TLR3 and CD55 have also been associated with influenza hospitalizations or death.[180]

Therapies designed to reduce the cytokine response have been beneficial in severe influenza in animal models,[181] although this has not been tested rigorously in humans. However, a meta-analysis suggested that steroid therapy for severe influenza in humans is associated with increased mortality.[182] Commonly used statins also have antiinflammatory properties, and an observational study suggested that use prior or during hospitalization may be associated with decreased mortality.[183] Because these drugs are very common, it has been suggested that they may be useful in a pandemic response.[184]

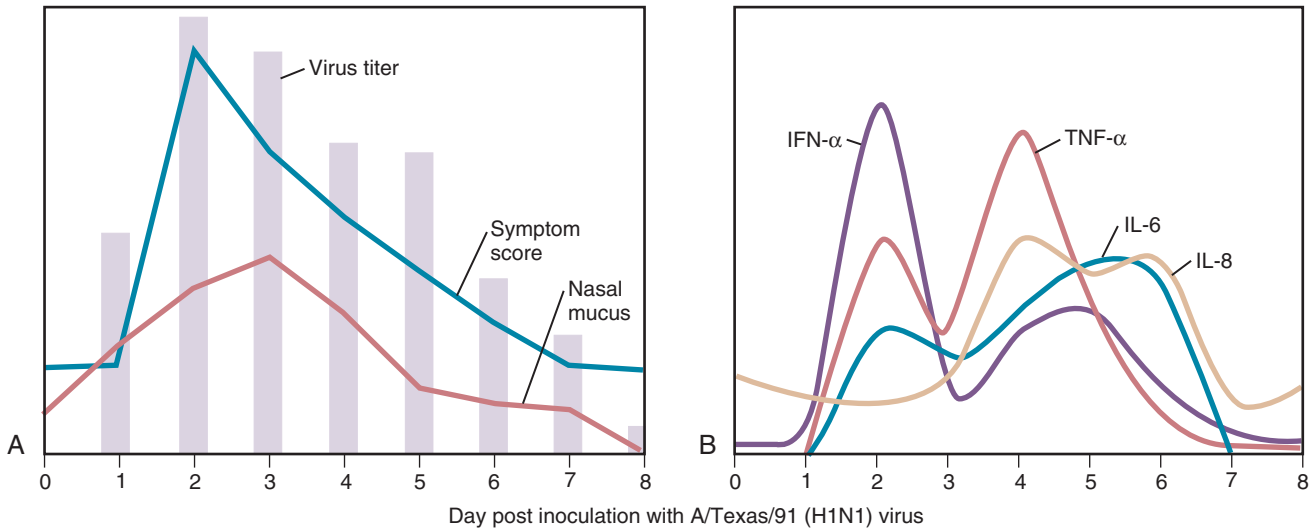

FIG. 165.7 Time course of virus shedding, symptoms, and cytokine responses of healthy adults after experimental inoculation with wild-type A/Texas/36/91 virus by nasal drops. (A) Mean $\log_{10}$ virus titer (tissue-culture infective dose [$TCID_{50}$] per milliliter of nasal secretions), clinical symptom scores, and nasal mucus weights (in grams). (B) Nasal cytokine levels measured by enzyme-linked immunosorbent assay (ELISA) (pg/mL lavage fluid, corrected for collection efficiency). In both graphs, multiple measurements have been combined for illustration, so that the *y*-axes are relative values only. Peak values reported in each assay are approximately as follows: virus titer, 3.6 $\log_{10}$ $TCID_{50}$/mL nasal secretions; symptom score, 7.0; nasal mucus weight, 7.0 g; interleukin (IL)-6, 450 pg/mL; interferon *(IFN)*-α, 150 pg/mL; tumor necrosis factor *(TNF)*-α, 270 pg/mL; IL-8, 9000 pg/mL. *(Data from Hayden FG, Fritz R, Lobo MC, et al. Local and systemic cytokine responses during experimental human influenza A virus infection: relation to symptom formation and host defense.* J Clin Invest. *1998;101:643–649.)*

Histopathology

Bronchoscopy of individuals with typical, uncomplicated acute influenza has revealed diffuse inflammation of the larynx, trachea, and bronchi, with mucosal injection and edema.[185,186] Biopsy in these cases has revealed a range of histologic findings, from vacuolization of columnar cells with cell loss, to extensive desquamation of the ciliated columnar epithelium down to the basal layer of cells (Fig. 165.8).[186] Individual cells show shrinkage, pyknotic nuclei, and a loss of cilia. Viral antigen can be demonstrated in epithelial cells.[187] In general, the tissue response becomes more prominent as one moves distally in the airway.[186] Epithelial damage is accompanied by cellular infiltrates primarily composed of lymphocytes and histiocytes. Histologic findings on autopsy in more severe cases show extensive necrotizing tracheobronchitis, with ulceration and sloughing of the bronchial mucosa,[188] extensive hemorrhage, hyaline membrane formation, and a paucity of PMN infiltration (Fig. 165.9). Patients with secondary bacterial pneumonia have the changes characteristic of bacterial pneumonia in addition to the tracheobronchial findings of influenza (Fig. 165.10). Recovery is associated with rapid regeneration of the epithelial cell layer and with pseudometaplasia.

Pathophysiology

Abnormalities of pulmonary function are frequently demonstrated in otherwise healthy, nonasthmatic young adults with uncomplicated (nonpneumonic) acute influenza. Demonstrated defects include diminished forced flow rates, increased total pulmonary resistance, and decreased density-dependent forced flow rates consistent with generalized increased resistance in airways less than 2 mm in diameter,[189,190] along with increased responses to bronchoprovocation.[189] In addition, abnormalities of carbon monoxide diffusing capacity[191] and increases in the alveolar-arterial oxygen gradient[192] have been seen. Of note, pulmonary function defects can persist for weeks after clinical recovery. Influenza in asthmatics[193] or in patients with chronic obstructive disease[194] may result in acute declines in forced expiratory vital capacity (FVC) or forced expiratory volume in 1 second (FEV_1). Individuals with acute influenza may be more susceptible to bronchoconstriction from air pollutants such as nitrates.[195]

Primary viral pneumonia occurs when virus infection reaches the lung either by contiguous spread from the upper respiratory tract or by inhalation. The trachea and bronchi contain bloody fluid, and the mucosa is hyperemic.[150] Tracheitis, bronchitis, and bronchiolitis are seen, with loss of normal ciliated epithelial cells. Submucosal hyperemia, focal hemorrhage, edema, and cellular infiltrate are present. The alveolar spaces contain varying numbers of neutrophils and mononuclear cells admixed with fibrin and edema fluid. The alveolar capillaries may be markedly hyperemic with intraalveolar hemorrhage. Acellular hyaline membranes line many of the alveolar ducts and alveoli.[150] Pathologic findings seen at biopsy of lung in nonfatal cases are similar to those described in fatal cases.[196]

The pathophysiology of bacterial superinfection has been studied intensively,[197] and a number of factors have been identified that could play a role. Uncomplicated influenza is associated with significant abnormalities in ciliary clearance mechanisms,[198] resulting in reduced clearance of bacteria from epithelial surfaces.[199] Alterations in PMNs and mononuclear cells may also contribute to enhanced bacterial infection. In mouse models, the inflammatory response to influenza, particularly the type I IFN response, also contributes to the downregulation of host antibacterial defense mechanisms.[200,201] The cytokine IL-10 plays an important role in this regard.[202] Influenza infection also reduces clearance by alveolar macrophages[203,204] and weakens NK responses.[205] In humans with H1N1 influenza, the development of pneumonia was associated with an early increase in T-regulatory cells (Tregs) in peripheral blood.[206] All of these observations are consistent with the concept that the inflammatory response to influenza infection leads to disruption in the normal host defense against bacterial pathogens, although direct demonstration of this in humans is lacking.

Host Response

Infection with influenza virus induces both systemic and local antibody, in addition to $CD4^+$ and $CD8^+$ T-cell responses, each of which plays a role in recovery from infection and resistance to reinfection. Epidemiologic and experimental observations in humans have shown that infection with influenza virus results in long-lived resistance to reinfection

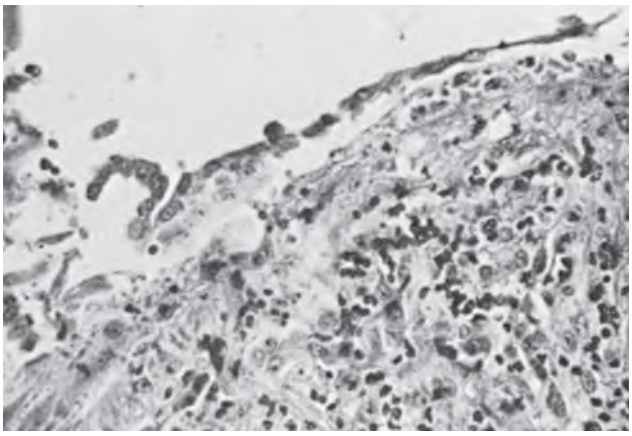

FIG. 165.8 A small bronchus in acute influenza A infection shows ulceration and attempted regeneration of epithelium. (Hematoxylin-eosin stain, ×100.) *(Courtesy I.D. Stuard, Reading, PA.)*

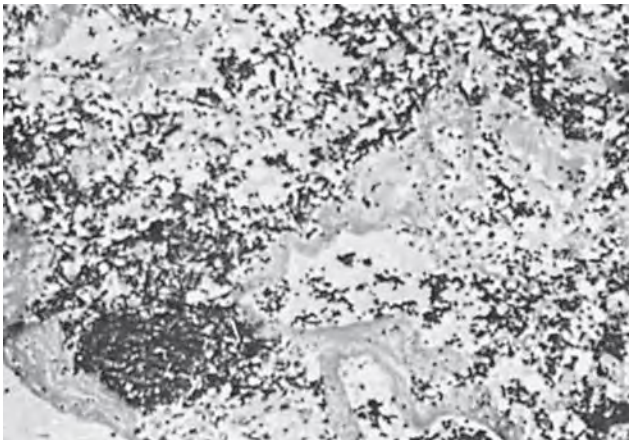

FIG. 165.9 Lung parenchyma in primary influenza viral pneumonia shows extensive hemorrhage, acellular hyaline membrane lining alveolar ducts and alveoli, and a paucity of inflammatory cells within the alveoli. (Hematoxylin-eosin stain, ×400.) *(Courtesy I.D. Stuard, Reading, PA.)*

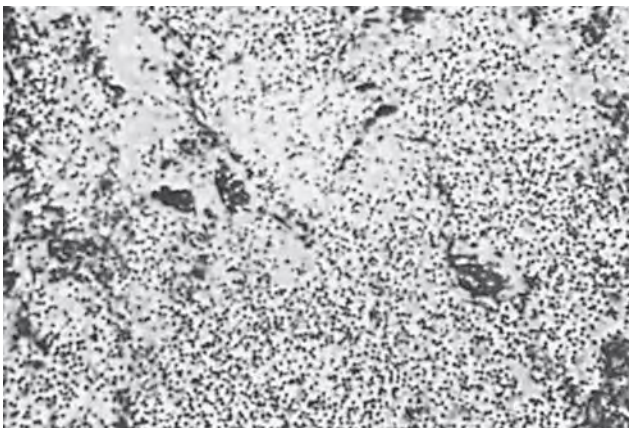

FIG. 165.10 Lung parenchyma in secondary bacterial infection *(Streptococcus pneumoniae)* complicating influenza A virus infection. Note the marked intraalveolar polymorphonuclear cell exudate. (Hematoxylin-eosin stain, ×400.) *(Courtesy I.D. Stuard, Reading, PA.)*

with the homologous virus. In addition, variable degrees of cross-protection within a subtype have been observed. Some epidemiologic data have raised the possibility of heterosubtypic protection in humans,[207] and identification of a variety of potentially cross-protective immune mechanisms is being explored as a strategy to increase the breadth of protection afforded by vaccination (see later).

Antibody Responses

Systemic Antibody Responses

Infection with influenza virus results in the development of antibody to the influenza virus envelope glycoproteins HA and NA, and to the structural M and NP proteins. Some individuals may also develop antibody to the M2 protein.[208] As measured with enzyme-linked immunosorbent assay (ELISA), serum IgM, IgA, and IgG antibody to the HA appear simultaneously within 2 weeks of inoculation of virus. The antibody response is more rapid after reinfection. The development of anti-NA antibodies parallels that of anti-HA antibodies.[209] Peak antibody responses are seen at 4 to 7 weeks after infection and decline slowly thereafter; titers can still be detected years after infection even without reexposure.[210]

Antibody to the HA can prevent attachment of the virus to the cell, and neutralizes virus infectivity.[211] A surrogate measurement of the neutralizing activity of serum is the hemagglutination-inhibition (HAI) assay, in which sera is tested for the ability to prevent agglutination of red blood cells by a virus preparation. An increased risk of laboratory-documented influenza among those with the lowest titers of preexposure HAI or neutralizing antibody is a consistent finding of most but not all studies. However, there is considerable uncertainty about the actual level of HA antibody that is the best predictor of protection, with estimates ranging from HAI titers of 1:8 to 1:160 or higher.[212] Given the substantial variation from laboratory to laboratory in the estimation of the HAI titer on the same set of samples,[213] the inability to use an absolute value for protection is not unexpected. In addition, the amounts of antibody needed to mediate protection could vary by population, degree of exposure, age, and specific influenza type or subtype, although this has not been analyzed comprehensively.

Studies evaluating the B-cell response of patients infected with novel influenza viruses, such as H5N1 or primary infections with pandemic H1N1, have identified B cells directed against epitopes on the stalk of the HA molecule.[214,215] The monoclonal antibodies produced by these B cells are typically highly cross-reactive among HA molecules within the same group; for example, group 1 specific antibodies react with H1, H2, and H5 viruses,[216] and group 2 specific antibodies react with H3 and H7 viruses.[217] Some levels of stalk-specific antibody are present in the sera of most adults, and the role of these antibodies in protection in humans has not been defined. However, there is considerable interest in exploiting this observation in the development of broadly cross-protective influenza vaccines.[218]

Antibody to the NA can be measured with NA inhibition or ELISA. In contrast to anti-HA antibody, anti-NA antibody does not neutralize virus infectivity but instead reduces efficient release of virus from infected cells, resulting in decreased plaque size in in vitro assays[219] and in reductions in the magnitude of virus shedding in infected animals.[220,221] Observations of the relative protection of those with anti-N2 antibody during the A/Hong Kong/68 (H3N2) pandemic,[209,222] and in experimental challenge studies in humans,[223,224] have shown that anti-NA antibody can be protective against disease and results in decreased virus shedding and severity of illness, but that it is infection permissive.[225]

Antibody to other influenza viral proteins have also been evaluated for potential protection. Antibody to M2 reduces plaque size in vitro, and passive transfer studies in mice have also suggested that antibody to the M2 protein of influenza A viruses may be partially protective if present in large enough amounts.[226] The mechanism of protection in vivo is related to mediation of antibody-dependent cytotoxicity.[227] Antibody to internal viral proteins such as M or NP are also cross-reactive among type A viruses, but they are nonneutralizing. Studies in mice have suggested that such nonneutralizing but cross-reactive antibody may mediate protection under some circumstances.[228] The mechanism by which antibody to viral proteins that are not exposed on the surface can mediate protection is unclear.

Mucosal Antibody Responses

The majority of studies of mucosal responses to influenza in humans have concentrated on measurement of HA responses with ELISA or neutralization tests, because nonspecific inhibitors of hemagglutination present in nasal mucus interfere with the standard HAI test. These studies have demonstrated significant mucosal responses to infection with wild-type virus or live-attenuated influenza vaccines (LAIVs). Both IgA and IgG are found in nasal secretions. Nasal HA-specific IgG is predominantly IgG1, and its levels correlate well with serum levels of HA-specific IgG1, suggesting that nasal IgG originates by passive diffusion from the systemic compartment.[229] Nasal HA-specific IgA is predominantly polymeric and IgA1, suggesting local synthesis.[230]

Studies in mice and ferrets have emphasized the importance of local IgA antibody in resistance to infection, particularly in protection of the upper respiratory tract. Polymeric IgA was shown to be specifically transported into the nasal secretions of mice and to protect against nasal challenge. Protection could be abrogated by intranasal administration of antiserum against IgA but not IgM or IgG.[231] Local antibody has also been shown to play a role in protection against antigenic variants in mice.[232] Studies in humans have also suggested that the resistance to reinfection induced by virus infection is mediated predominantly by local HA-specific IgA, whereas that induced by parenteral immunization with inactivated virus depends also on systemic IgG.[233] Important to note, either mucosal or systemic antibody alone can be protective if present in high enough concentrations, and optimal protection occurs when both serum and nasal antibodies are present.[234,235]

Cellular Responses

The induction of cellular immune response to influenza virus infection has been studied intensively in murine models, and such studies suggest that B cell, $CD4^+$ T cell, and $CD8^+$ T-cell responses all can play a role in protection against disease and recovery from infection. A large number of HLA class I restricted ($CD8^+$ T cell) and HLA class II restricted ($CD4^+$ T cell) epitopes have been described, and in situations where those epitopes are on relatively well conserved influenza proteins such as the polymerase, NP, and M proteins, the cellular responses are cross-reactive between subtypes, although not between types A and B.

Cellular immune responses to influenza vaccination and infection have not been studied as extensively in humans, but both B-cell (memory B-cell and antibody-secreting cell [ASC]) and $CD4^+$ T-cell and $CD8^+$ T-cell responses in peripheral blood have been described after infection or vaccination.[236] It can be difficult to capture the peak of the response, and detectable increases in antigen-specific cells may be seen on only a few days after exposure. In general, the peak cellular response occurs somewhere between 5 and 14 days depending on the status of the subject and the nature of the response. As seen in murine models, a major component of the cellular response is directed at conserved peptides to which the subject has already been exposed during previous infections or vaccinations.

ASCs appear in blood and tonsils as early as 2 days after vaccination, and are detected in the blood of adults and older children more frequently than in young children after immunization.[237] An increase in cytotoxic T lymphocytes, directed primarily at the conserved internal proteins, has been shown in healthy adults with a peak at 14 and 21 days after vaccination and return to baseline at 6 months. An increase in HA-specific $CD8^+$ T cells on day 7 after vaccination has also been detected by means of tetramer staining in adults receiving inactivated influenza vaccine (IIV).[238]

An important role of the cellular immune response in recovery from influenza infection in humans is strongly supported by the observation of prolonged illness and viral shedding in individuals who are lymphopenic as a result of disease or chemotherapy.[239] However, it has been difficult to develop specific markers of T-cell immunity as correlates of protective immunity. Activated T cells, in the form of granzyme B–positive T cells have been associated with protection in older subjects.[240] In the human challenge model, early studies identified virus-specific cytotoxic T cells as correlated with reductions in the duration and level of virus replication in adults.[241] In a subsequent study done many years later by the same group, prechallenge $CD4^+$ T cells, but not $CD8^+$ T cells, correlated with relatively lower levels of viral shedding and

symptoms after experimental infection.[242] In a large study of the efficacy of live-attenuated vaccine in children, it was shown that the presence of >200 influenza-specific T cells assayed by IFN-γ ELISPOT was associated with a decreased risk of PCR-documented influenza.[243]

In community surveillance of healthy adults during the pandemic of 2009, and in a setting of low baseline antibody to the pandemic virus, $CD8^+$ T cells were strongly correlated with protection against severe illness.[244] Under similar circumstances, the presence of NP-specific, predominantly $CD8^+$ T cells was correlated with less symptomatic influenza.[245] In addition, an early $CD8^+$ T cell response is associated with successful recovery from severe H7N9 illness in humans.[246] The development of more sophisticated markers that can specifically identify reactive cells in peripheral blood will help to define the role of cellular immunity in protection, but the field remains limited by the lack of convenient access to compartments other than the peripheral blood in humans.

DIAGNOSIS

The majority of cases of influenza are diagnosed on clinical grounds. In selected circumstances as discussed here, the diagnosis of influenza can be confirmed with laboratory diagnostic tests in which significant advances have been recently made in sensitivity and availability. These approaches are described in the following sections.

Clinical Diagnosis

Most cases of influenza are diagnosed based on compatible clinical symptoms and seasonal epidemiology. That is, when the presence of influenza virus is confirmed in a region or community, healthy adults with acute influenza-like illness most commonly have influenza. In fact, several studies have shown that the accuracy of a clinical diagnosis in healthy adults in the setting of an influenza outbreak is as high as 80% to 90%.[247–249] In an analysis of symptoms in young adults being assessed for entry into studies of influenza virus treatment, the best multivariate predictors of laboratory-confirmed influenza virus infection were cough and fever,[248] with an increasing predictive value with increasing levels of fever. However, the predictive value of such a symptom complex may be less in older adults[250] and in children.[251] In nursing homes, the presence of cocirculating pathogens (such as respiratory syncytial virus) that can result in identical symptoms can clearly complicate the ability to make a clinical diagnosis of influenza specifically.[252,253]

Laboratory Diagnosis

A wide variety of laboratory diagnostic approaches have been developed and are reviewed here. Available diagnostic testing information is updated frequently by the CDC on the website http://www.cdc.gov/flu/professionals/diagnosis/table-testing-methods.htm.

Polymerase Chain Reaction–Based Tests

The most widely used method in clinical laboratories to detect influenza infection is PCR tests. PCR-based tests have the advantage of being potentially more sensitive than other available tests and allow detection in samples in which the virions have lost viability. In addition, it is possible to devise multiplex techniques so that a single test can detect a number of different agents,[254] and many PCR methods also allow rapid subtyping of the virus. For these reasons, PCR and particularly real-time reverse-transcriptase polymerase chain reaction (rtRT-PCR) has become the gold standard of influenza diagnostic testing and is generally available at clinical laboratories. Diagnostic sensitivities of the tests depend on the level of virus present in various specimens, and generally samples obtained by nasopharyngeal swabs have slightly higher sensitivity, similar to the findings with antigen detection tests. In some patients with lower respiratory tract disease, sputum samples or tracheal aspirates have been positive when nasal swabs were not. Swabs with wooden handles should be avoided for PCR diagnostic testing, because these can interfere with the chemistry of the assay.

Improvements have been made in PCR techniques that have reduced the time to carry out assays in the laboratory to as little as 45 to 80 minutes. However, specimens still require transport from the point of care to the laboratory. Thus, despite their high accuracy and sensitivity, this may limit their usefulness for patient management. Therefore there has been considerable effort to develop simple, rapid nucleic acid–based detection methods that could be used at the point of care.

Rapid Nucleic Acid Amplification Tests

An alternative method of nucleic acid detection is isothermal amplification, which does not require a thermal cycler. A variety of isothermal amplification techniques have been developed, with the most frequently used approach being loop-mediated isothermal amplification (LAMP).[255] These assays are highly accurate, on a par with PCR-based tests, and are rapid, giving results in less than 30 minutes.[256] Because they are simple to operate, they can be approved for use at the point of care, which greatly improves their potential usefulness. However, these assays are currently expensive and not generally designed to identify other respiratory viruses.

Rapid Influenza Diagnostic Tests

The tests widely referred to as "rapid diagnostic" tests are based on immunologic detection of viral antigen in respiratory secretions. In this approach, the sample is treated with a mucolytic agent and then tested with specific antibody that results in a color change or similar end point that is read visually. All of these rapid tests are relatively simple to perform and can provide results within 30 minutes; many are eligible for Clinical Laboratory Improvement Amendments (CLIA) waiver and can be performed at the point of care. The reported sensitivities of each test in comparison to cell culture have ranged between 40% and 80%, and they are somewhat dependent on the nature of the samples tested and the patients from whom they were derived. In general, sensitivities in adults and older adult patients tend to be lower than those reported in young children, who shed much larger quantities of virus in nasal secretions and therefore have much higher concentrations of antigen in their samples. Similarly, sensitivity is likely to be higher early in the course of illness, when viral shedding is maximal. Although all types of respiratory samples can be used in such tests, the sensitivity appears to be better with nasopharyngeal swabs and aspirates than with throat swabs or gargles.[257,258] In some tests, the use of a digital readout can improve sensitivity compared with visual inspection.[259] However, because of the relatively low sensitivities of these tests, their usefulness in clinical decision making may be limited.

Virus Isolation

Isolation of influenza virus in cell culture has historically been the definitive laboratory test to diagnose influenza infection. Virus can be isolated readily from nasal swab specimens, throat swab specimens, nasal washes, or combined nose and throat swab specimens, or sputum samples. Samples should be placed into containers of viral transport medium and transported to the laboratory as soon as possible. Virus can be detected in a variety of cell lines by means of cytopathic effects, hemadsorption, or immunologic testing. Over 90% of positive cultures can be detected within 3 days of inoculation[260] and the remainder by 5 to 7 days. However, virus isolation requires specialized expertise and equipment and does not provide diagnosis within the time frame of clinical decision making.

Role of Viral Diagnosis in Clinical Decision Making

Most cases of influenza, occurring in otherwise healthy individuals with typical symptoms during the course of a recognized influenza epidemic, do not need specific viral confirmation. However, diagnostic testing should be used if the results of the test will influence subsequent clinical management. This would include decisions regarding the use of antiviral agents, the need for antibacterial drugs, and considerations for infection control.[261]

During the influenza season, certain groups should be considered for diagnostic testing if the results will influence decision making. Outpatients with higher risk for influenza complications (see Table 165.6) should be considered for testing if they are presenting within 5 days of symptom onset. Testing beyond this window may not be useful because the yield would be low and the patient would be outside the window for antiviral therapy. In contrast, outpatients who are immunocompromised, elderly, or infants may have prolonged shedding and could be considered for testing even beyond the 5-day window.

Hospitalized patients with febrile illness during influenza epidemics should also be tested regardless of duration of symptoms, as should patients who develop febrile illnesses in hospital during influenza epidemics, to determine nosocomial transmission. In addition, patients who are not in one of these groups might undergo diagnostic testing as part of epidemiologic surveillance in the community.

Outside of influenza epidemics, diagnostic testing should usually be restricted to patients with influenza-like symptoms who are epidemiologically linked to suspected outbreaks of influenza. Examples would include travelers returning from a region with influenza activity or individuals exposed during institutional outbreaks.

The choice of diagnostic test strategy will depend on local practice and availability of specific tests. Interpretation of rapid diagnostic test results, and, to a lesser extent, molecular assays, should take into consideration the known sensitivity and specificity of the test, and the a priori likelihood of influenza. Most positive results of rapid diagnostic tests obtained outside of the influenza season will be false positives. Important to note, if influenza is strongly suspected in a high-risk patient, antiviral therapy should be initiated without waiting for the results of diagnostic testing.

PREVENTION

Currently Available Vaccines

Multiple forms of influenza vaccine are currently available both in the United States and elsewhere, including virion- or protein-based IIVs administered intramuscularly, and LAIVs administered intranasally. Both types of vaccines are thought to work primarily by inducing antibody against the viral hemagglutinin, although other mechanisms probably also play a role.

As described earlier, influenza viruses undergo continual antigenic evolution to escape from prior immunity. As a result, the match between the viruses chosen as components of the vaccine and the viruses that circulate during the season is an important determinant of the efficacy of that season's vaccine.[262] Successfully matching the vaccine to the epidemic requires a considerable effort devoted to worldwide surveillance and subsequent antigenic characterization of emerging new variants. A final decision regarding strain selection for next year's vaccine generally must be made at least 6 months before expected vaccination—that is, in February for the Northern Hemisphere and September for the Southern Hemisphere. The need to constantly update vaccine formulations and to readminister vaccine on a yearly basis represents some of the biggest challenges in effectively controlling the impact of influenza.

The composition of the vaccine has changed over time to reflect changes in the epidemiology of influenza viruses. Since 1977, influenza vaccines have contained three strains representing the most up-to-date antigenic variants of A/H3N2, A/H1N1, and B viruses. Since approximately 2004, two antigenically distinct lineages of influenza B viruses, the Victoria lineage and the Yamagata lineage, have cocirculated. Therefore, quadrivalent formulations of vaccine containing both lineages were licensed beginning in the 2013–2014 influenza season. The nomenclature of influenza vaccines has changed to reflect this difference, with trivalent preparations now termed inactivated influenza vaccine-3 (IIV-3) and quadrivalent IIV-4.

Each of the vaccine types currently available for prevention of influenza are described briefly here.

Egg-Grown Inactivated Influenza Vaccines (IIV-3, IIV-4)

The original influenza vaccine, which consisted of formalin-inactivated whole virions grown in embryonated chicken eggs, was demonstrated to have a protective efficacy of 70% in healthy adults[9] and was licensed in 1945. Since then, although there have been several important advances in the techniques for producing vaccine, the basic vaccine strategy has remained the same. The development of the zonal gradient centrifuge allowed more efficient production and more highly purified vaccines from which reactogenic contaminants had been removed. Treatment of the whole virus with solvents to create "split" vaccines, or with detergents to create "subunit" vaccines, resulted in a vaccine with fewer adverse reactions, particularly fever, than the whole-cell vaccine. The efficiency of vaccine production was also improved through the development of techniques to create reassortant viruses adapted to provide high yield from hens' eggs.[263] Since the late 1970s, egg-grown vaccines have been standardized to contain at least 15 μg of each hemagglutinin (HA) antigen as assessed with single radial immunodiffusion (SRID) using antibody reagents prepared against the components of the vaccine. Embryonated hens' eggs are an extremely efficient substrate for the growth of most influenza viruses, and consequently egg production remains the primary mode of production for current influenza vaccines. However, although eggs are an efficient substrate for vaccine production, the process of adapting to growth in an avian substrate can result in mutations in the hemagglutinin protein that result in significant antigenic differences from the original starting material and adversely affect vaccine performance in unpredictable ways.[264–267]

Cell Culture–Derived Inactivated Vaccine (ccIIV-4)

One strategy to avoid the problems associated with egg adaptation would be to produce the vaccine by propagating the viruses in mammalian cell culture. Currently, a vaccine produced in a qualified line of Madin-Darby canine kidney (MDCK) cells is licensed for use in individuals aged 4 years and older in the United States. Other cell lines such as Vero cells and PerC.6 cells have also been explored for influenza vaccine production.

Recombinant Hemagglutinin Expressed in Insect Cells (rIV-4)

Another alternative is to express the hemagglutinin using an appropriate expression system. The expression of proteins in insect cells using recombinant baculovirus expression vectors can be achieved rapidly and results in proteins with mammalian-like glycosylation. A recombinantly expressed hemagglutinin vaccine is currently licensed in the United States for ages 18 and above. This vaccine uses a higher dose of HA antigen per component (45 μg), although the reagents used for release criteria are different from those used for egg-derived vaccine.

High-Dose Egg-Grown Inactivated Vaccine (HD-IIV3)

It has long been recognized that the immune response to influenza vaccine may be decreased in older adults. The use of higher doses of vaccine may be able to circumvent this problem, and a vaccine that uses four times the usual dose (60 μg per component) has been developed and shown to induce higher levels of antibody and improved protection against H3N2 viruses in older adults. The vaccine is produced in eggs and contains a single B-virus lineage, and is licensed for use in adults aged 65 and older.

MF-59 Adjuvanted Inactivated Influenza Vaccine (aIIV-3)

Another approach to improving vaccine performance is the use of adjuvants, or immune stimulators designed to improve the response to a coadministered antigen. The squalene-based oil-in-water adjuvant MF59 has been shown to increase the immunogenicity of egg-derived influenza vaccine in older adults, and has been licensed in the United States and elsewhere in this age group. MF59 adjuvanted vaccine is also currently being evaluated for potential improved efficacy in children.

Intranasal Live-Attenuated Influenza Vaccine (LAIV-4)

Live-attenuated vaccines have a long track record of success against a wide variety of viral diseases, such as smallpox, measles, polio, and others, in part because they generate a diverse immune response that mimics the immune response to the pathogen. Developing live vaccines for influenza represents a special challenge because of the frequent antigenic changes in the virus. Creation of reproducibly attenuated antigenically variant LAIVs takes advantage of the principle of reassortment to generate attenuated vaccines for new antigenic variants using a vaccine master donor virus (Fig. 165.11). The master donor viruses used in the LAIV currently licensed in the United States are the cold-adapted influenza A/Ann Arbor/6/60 (H2N2) and B/Ann Arbor/1/66 viruses, developed by Dr. John Maassab at the University

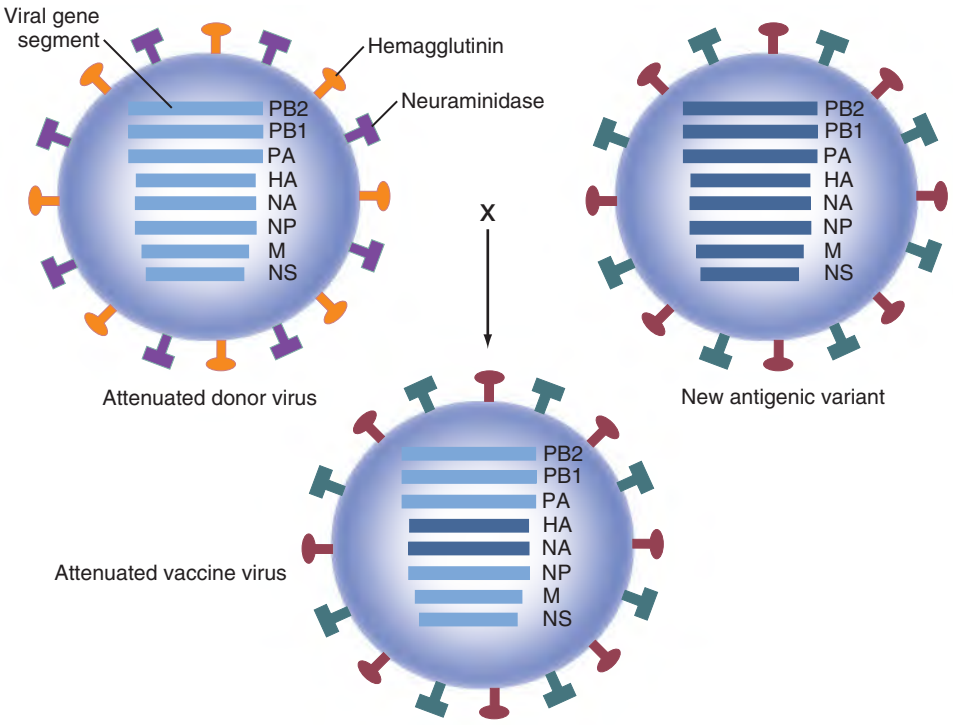

FIG. 165.11 Genetic reassortment is used to generate new live-attenuated vaccine viruses. The genetic basis of attenuation of the "master donor virus" is encoded in gene segments other than hemagglutinin (HA) or neuraminidase (NA). Using either mixed infection in cell culture or reverse genetics techniques, the genes encoding HA and NA of new antigenic variants can be inserted into the background of the master donor virus to rapidly create a new attenuated vaccine virus.

of Michigan in the 1960s.[268] A quadrivalent formulation of these viruses (LAIV-4) is currently licensed in the United States and other countries for use in individuals ages 2 to 49. A second type of LAIV, based on the cold-adapted A/Leningrad/60 and B/Leningrad/66 master donor viruses, is also licensed in some countries.

Safety

Inactivated Vaccines

Hundreds of millions of doses of IIV are administered to adults, elders, and children each year, and the safety of these vaccines has been repeatedly confirmed. For example, no increase in clinically important medically attended events has been noted among over 251,000 children <18 years of age who were enrolled in one of five health maintenance organizations within the Vaccine Safety Datalink, the largest published postlicensure population-based study of vaccine safety.[269] The most common adverse events reported after immunization with IIV are tenderness and/or pain at the injection site. Most injection site reactions are mild and rarely interfere with daily activities. Systemic reactions after immunization of adults with inactivated vaccine are uncommon. In placebo-controlled clinical trials in younger and elderly adults, rates of systemic reactions were similar among groups given inactivated vaccine or placebo.[270,271]

Immediate hypersensitivity reactions (hives, wheezing, angioedema, or anaphylactic shock) after vaccination with inactivated vaccine can also occur, and vaccine is considered contraindicated for persons who experienced a previous anaphylactic reaction following vaccine administration. However, recent studies support the safety of IIV among persons who experience mild allergic reactions to eggs, such as hives.[272] Current recommendations[273] for persons with other forms of egg allergy such as history of angioedema or respiratory distress are that any inactivated vaccine may be used, but vaccine should be administered in a medical setting where emergent severe allergic reactions can be treated.

Guillain-Barré syndrome (GBS), an acute inflammatory demyelinating polyneuropathy, was associated with the 1976 swine influenza vaccination campaign, with an increased risk of approximately 1 per 100,000 vaccinees.[274] Subsequent studies have suggested a statistically significant but very slight increased relative risk of GBS within 7 weeks of influenza vaccination.[275] A meta-analysis of 39 observational studies conducted from 1981 to 2014 confirmed a slightly increased risk of GBS after influenza vaccination.[276]

The oculorespiratory syndrome (ORS) is a syndrome of red eyes, facial edema, and/or respiratory symptoms such as coughing, wheezing, sore throat, hoarseness, difficulty breathing, or chest tightness that develop within 2 to 24 hours after vaccination, associated with a specific influenza vaccine used in Canada, but not elsewhere.[277] The specific mechanism underlying this phenomenon is unknown.

Pregnancy is recognized as a risk factor for more severe influenza, and pregnant women are an important target group for immunization. Most studies of pregnancy outcomes have found no association between vaccination and adverse pregnancy outcomes, although relatively less information is available regarding first trimester vaccination. Influenza vaccine is currently considered safe in all trimesters.[278]

During the response to the 2009 pandemic, monovalent H1N1 inactivated vaccines were administered in several countries adjuvanted with the squalene-based adjuvant AS03. The use of AS03 with pandemic H1N1 vaccine was associated with an increased risk of narcolepsy during vaccine campaigns in Europe. Initially noted in Finland,[279] this association has been established in multiple other locations where AS03-adjuvanted pH1N1 vaccines were used routinely in young children.[280,281] The pathogenesis of this disorder involves presumably immune destruction of neurons in the hypothalamus responsible for synthesis of the neurotransmitter hypocretin.[282] The mechanism that might link the use of influenza vaccine to the development of narcolepsy is unclear. Almost all cases that have been HLA typed are positive for the narcolepsy risk allele DQB1*0602.[283] An increased incidence of narcolepsy was also reported after H1N1 infection in China,[284] suggesting that narcolepsy may be linked in some way to the specific antigen in the vaccine. Narcolepsy after influenza vaccination has not been reported in other countries[285] and it was not reported after use of influenza vaccines containing the similar squalene-based adjuvant MF59.[286] However, the age distribution of adjuvant vaccine use also differed among countries and vaccines, and the role and potential mechanisms of AS03 in narcolepsy remain undetermined.

Live-Attenuated Vaccines

LAIVs based on the cold-adapted A/Ann Arbor and B/Ann Arbor viruses display three characteristic phenotypes: effective replication at low temperatures (25°C), reduced replication at high temperatures (38°C–39°C), and attenuation in a variety of animal models.[287] Multiple mutations in the internal gene segments contribute to these phenotypes.[288] Studies using single gene reassortants demonstrated that at least three of these gene segments (PB1, PB2, and PA) independently participate in attenuation in both animals and human subjects.[289,290] For the influenza B/Ann Arbor virus, the PA, PB2, NP, and M gene segments contribute to the cold-adapted and attenuation phenotypes[291,292] Because both donor viruses are attenuated at multiple sites, it would be predicted that the vaccines should be phenotypically stable even after prolonged replication in seronegative children, and this has been shown in clinical studies.[293]

Cold-adapted LAIVs have been well tolerated in adults, with mild nasal symptoms and sore throat occurring at rates slightly in excess of those in placebo recipients. These vaccines have also been shown to be safe and well tolerated in children, although children younger than 8 years have had slightly increased but variable rates of low-grade fever, runny nose, and abdominal symptoms in the 7 days after vaccination compared with placebo recipients. However, when considering all the pediatric studies in aggregate, no consistent symptom was significantly more common in LAIV recipients compared with placebo recipients. In older children, 11 to younger than than 16 years, sore throat was observed slightly more frequently in LAIV recipients than in recipients of inactivated influenza.

In larger studies, wheezing has been consistently identified as a vaccine-associated side effect in young children, although occurring at low rates. In the largest trial, medically significant wheezing within 42 days of vaccination was reported in 3.8% of children <2 years old after receipt of LAIV compared with 2.1% in those who received IIV.[294] Wheezing generally occurs in the youngest, previously unvaccinated children after the first dose of vaccine. Because of this observation, LAIV is currently approved for use in the United States for children ≥2 years old who do not have a history of asthma.

Safety of LAIV has also been demonstrated in some high-risk patient groups. No significant vaccine-related adverse events were seen in studies of children with cystic fibrosis or asthma, and vaccinated children with asthma did not experience significant changes in FEV_1, use of β-adrenergic rescue medications, or asthma symptom scores compared with placebo recipients.[295] LAIV has also been well tolerated in adults with chronic obstructive airway disease.[296,297] and older adults,[298] although the vaccine is not recommended for use in these populations.

Young children with advanced HIV infection may have difficulty clearing wild-type influenza virus from the respiratory tract, and there have been several reports of very prolonged virus shedding in highly immunosuppressed children with AIDS. However, in small studies in adults[299] and children[300] with HIV, LAIV was well tolerated and not associated with prolonged shedding.

Transmission of LAIV to susceptible contacts does not appear to happen frequently. LAIV can be recovered from nasal secretions of about half of adult recipients, although generally shedding of LAIV by adults is of low titer and short duration.[301] Transmission of LAIV from vaccine recipients to susceptible contacts has been rarely detected in studies of young children involved in daycare-like settings. In the largest study, 197 children between 8 and 36 months of age in a daycare setting were randomized to receive LAIV or placebo, and LAIV was detected in one placebo recipient. The estimate of transmissibility in this age group was 0.6% to 2.0%.[302] However, because of the possibility of transmission, LAIV is not recommended for close contacts of individuals who have levels of immunocompromise that necessitate a protected environment.[273]

Immune Responses to Vaccination

Intramuscular Protein Vaccines

Increases in serum HAI antibody are seen in about 90% of healthy adult recipients of vaccine. Only a single dose of vaccine is required in individuals who were previously vaccinated or who experienced prior infection with a related subtype, but a two-dose schedule is required in unprimed individuals.[303,304] Primed individuals also generally respond with antibody that recognizes a broader range of antigenic variants than do unprimed individuals.[305] Serum antibodies peak between 2 and 4 weeks after vaccination but fall quickly, reaching near baseline before the next influenza season.[306] Despite evidence that NA-specific antibody responses contribute to protection, neither the NA content nor the enzymatic activity of the vaccine is standardized, and NA-specific antibody responses are not routinely assessed. Antibody responses to the NA develop, albeit at a lower rate than those to the HA.

Mucosal HA-specific antibody responses are generally not a major component of the response to parenterally administered inactivated vaccine.[307,308] However, dose-related increases in mucosal HA-specific antibody responses have been observed following IM immunization with increasing doses of a monovalent influenza A/H1N1 vaccine.[309]

HA-specific ASCs peak in peripheral blood peak approximately 7 to 8 days after IIV administration in adults.[310–312] ASCs may be less frequently detected in the blood of children.[237]

Cellular responses, including $CD4^+$ T-cell responses, are also noted after IIV,[313] and there is a strong correlation between the CD4 T-cell response and the antibody response.[314,315] Baseline frequencies of influenza-specific, IFN-γ–producing memory $CD4^+$ T cells are higher in children who received more previous vaccinations.[316] An increase in HA-specific $CD8^+$ T cells on day 7 after vaccination has also been detected by means of tetramer staining in adults receiving IIV.[238] Although the induction of HAI antibody is generally accepted as a correlate of vaccine protection, in some populations such as the elderly it has been suggested that induction of cellular responses may also correlate with protection.[317]

Unimmunized young children generally require two doses of IIV given at least 4 weeks apart to generate substantial serum antibody responses. Once a child has been primed with two doses of vaccine, a single dose of vaccine is recommended in subsequent seasons regardless of age. However, predicting immune responses of young children who have received single doses in two consecutive seasons is more complex. In studies performed in immunologically naïve children, responses among children who received the first dose of vaccine in the spring and the second dose in the fall were similar when compared with those who received both doses in the fall when vaccine antigens did not change.[318] However, in a second study done when the influenza B component changed between seasons, the response in children who received a dose in the spring and then an antigenically different influenza B in the fall was inferior to the response to two doses of the same B antigen in the fall.[319] Whereas most children 6 months of age or older respond to vaccine after receiving the recommended number of doses, antibody responses in infants are reduced compared with older children. Reduced responses among very young children may be related to a combination of immaturity of the immune system and a lower degree of priming.

When compared with younger adults, the proportions of elderly subjects who achieve a putative protective HAI antibody titer are lower. Both age and underlying conditions may contribute to these lowered responses. Unlike in children, the administration of a second dose of vaccine in elders is not associated with improved responses.[320] In some studies, the history of multiple prior vaccinations was better correlated to reduced vaccine responses than was age.[321] Although the dose-response curve for seasonal influenza vaccine is rather flat, the administration of a fourfold higher dose (60 μg/HA) is associated with an improved serum HAI response in elders.[322] High-dose vaccine has also been reported to induce significantly higher frequencies of response and higher levels of antibody to the NA in elders when compared with a standard dose.[323] The high-dose vaccine is licensed in the United States for use in adults older than 65.

The MF59 adjuvant is also associated with improved antibody responses in older adults. Although there is relatively little effect on the immune response to seasonal vaccines in healthy young adults,[324] the oil-in-water emulsion results in an approximately 50% increase in antibody titers in older adults,[325] and MF59 adjuvanted seasonal inactivated vaccines have been licensed for use in elders in Italy for several years. Recently, MF59 adjuvanted standard-dose inactivated vaccine was licensed for elders in the United States based on studies showing noninferiority of the immune response to that of standard-dose inactivated vaccine, although the adjuvanted vaccine was not superior.[326]

In addition, individuals on immunosuppressive therapy, those with renal disease, and transplant recipients have impaired responses to vaccination. To be maximally effective, immunizations should be given before transplantation, should avoid the nadir of white counts, and should include vaccination of close contacts.[327] In HSCT, these have included better responses in autologous compared with allogeneic transplants, the time since transplantation, the use of myeloablative therapy as opposed to reduced intensity, the presence of graft-versus-host host disease,[328] and the specific immunosuppressive chemotherapy being used. Solid organ transplant recipients have similarly decreased immune responses. In addition to the time since transplantation and the general state of immunosuppression, mycophenolate is associated with decreased responsiveness to influenza vaccine,[329,330] and sirolimus may associated with relatively better responses.[331] In both allogeneic hematopoietic stem cell transplant (HSCT) recipients and solid organ transplant types of transplant recipients, there is concern regarding whether the immune stimulus of vaccination, with or without an adjuvant, might nonspecifically stimulate increased severity of graft-versus-host disease or organ rejection. However, the weight of evidence does not support a significant association between vaccination and the development of autoimmune adverse events.

The responsiveness to influenza vaccination in HIV-infected individuals is related to the degree of immunosuppression.[332,333] Most patients with chronic lung disease respond reasonably well to vaccination, and steroids at doses commonly used to treat reactive airway disease do not appear to preclude vaccine responses.[334,335]

Intranasal Live-Attenuated Vaccine

Studies of the immunogenicity of cold-adapted reassortant vaccines have been carried out in children, adults, and older adults. The results of these studies are consistent with the hypothesis that the replication of cold-adapted vaccines in the upper respiratory tract, and hence their immunogenicity, is influenced by the susceptibility of the host at the time of vaccination. The frequency and magnitude of immune responses to vaccination are therefore highest in young children, intermediate in adults, and lowest in older adults who have been repeatedly infected with influenza viruses throughout their lifetime. In addition, the mucosally administered LAIV is generally more effective than parenterally administered IIV at inducing nasal HA-specific IgA, whereas inactivated vaccine usually induces higher serum titers of HAI and HA-specific IgG antibody.[336]

Most susceptible children demonstrate measurable serum and mucosal HA-specific antibody responses. Mucosal responses have been demonstrated in up to 85% of young children after LAIV.[337] In contrast, adults generally have a low rate of serum antibody response after LAIV,[338,339] and relatively lower rates of mucosal responses.[340] Even in those prescreened to have low prevaccination vaccine-specific influenza antibody, the rates of serum antibody responses to intranasal LAIV in adults and older adults are low.[339] Immune responses in elderly subjects are relatively rare.[341,342]

Influenza-specific IgA and IgG ASCs peak on days 7 to 12 after either LAIV or IIV in both adults and older children.[237] In contrast to children, IgG ASCs were significantly higher in adults after IIV than LAIV. Antibody responses were also significantly lower after LAIV than IIV in both adults and children, and in general the development of ASCs seemed to be a more sensitive indicator of vaccine take after LAIV than was antibody response. The levels of prevaccination memory B cells were low in all age groups, but numbers of prevaccination memory B cells were higher in adults than in children. IIV, but not LAIV, increased the numbers of circulating memory B cells at 1 month.

Influenza-specific IFN-γ producing $CD4^+$ and $CD8^+$ lymphocytes have also been detected after LAIV or IIV administration.[343,344] In children 5 to 9 years of age, IIV resulted in increases in the numbers of $CD4^+$ but not in the numbers of $CD8^+$ cells on day 10 after vaccination. There were no real changes in natural killer (NK) cells after IIV administration. In contrast, administration of LAIV resulted in increases in both IFN-γ–producing $CD4^+$ and $CD8^+$ cells, in addition to NK cells. In adults, there were no consistent changes in any subset after either IIV or LAIV, although there was much variability in the responses.

The mechanism of protection induced by cold-adapted vaccine has mostly been evaluated in experimental infection studies. Cold-adapted vaccine is protective in these experiments in the absence of significant serum HAI responses, suggesting that the main protective effect is induction of mucosal antibodies.[234,235] Finally, an analysis of data collected during a large field trial evaluation of LAIV concluded that the postvaccination number of influenza virus–specific IFN-γ–producing T cells was the best correlate of vaccine-induced protection.[243] However, no clear correlate of immune protection afforded by LAIV has been identified.

Markers of Immune Responsiveness

A number of methods have been developed that allow an extensive, unbiased assessment of the state of the immune system, which, combined with sophisticated bioinformatics analysis, has provided the opportunity to assess the genetic and metabolic networks involved in generating an immune response. For example, previous studies of inactivated vaccines suggested that genes in the IFN response pathway are highly upregulated in healthy males who mount the highest serum HAI responses to IIV, in comparison with those who have low or absent responses.[345] In another study done in both males and females, the PBMCs of responders collected on the day after vaccine were enriched for genes involved in cell-mediated and inflammatory responses.[346] Of interest, similar gene expression signatures have been identified as correlates of protective immunity induced by 17D yellow fever vaccine.[347]

Because the correlates of immunity induced by LAIV have not been clearly defined, gene signatures predictive of potentially protective immunity cannot be generated. However, vaccination of adults with seasonal LAIV was associated with upregulation of a variety of IFN-related genes, particularly on day 3 after vaccination,[346] suggesting that vaccine virus replication was required for induction. Upregulation of type 1 IFN responses have also been documented after LAIV in children.[348] Although these signatures may not allow prediction of vaccine efficacy, they may provide more sensitive indications of vaccine take than conventional measures of cell culture isolation and development of serum antibody responses.

Efficacy (Results of Randomized Prospective Studies)

The ability of influenza vaccines to prevent influenza has been assessed in numerous clinical studies that have varied greatly in design, populations, and end points. These studies have included prospective, randomized controlled studies, in which case they are referred to as efficacy studies, and a wide variety of nonrandomized cohort and retrospective studies that have assessed vaccine effectiveness (VE). End points evaluated in these studies have included both laboratory-confirmed influenza and non–laboratory-confirmed respiratory illnesses. In this regard, it has been recognized that studies that use a serologic definition of influenza infection may overestimate the efficacy of influenza vaccine, because it will be harder to demonstrate postvaccination to postseason antibody increases in the vaccinated group.[349]

Intramuscular Protein Vaccines

Randomized studies of inactivated vaccine efficacy against laboratory-confirmed influenza have mostly been conducted in healthy adults. These studies have shown a wide range of efficacy, from approximately 40% to 80%, with lower levels of efficacy typically seen in years with apparent antigenic mismatch. For example, the efficacy of IIV for preventing culture-proven influenza A illness in adults was 76% (95% confidence interval [CI], 58%–87%) for H1N1 and 74% (95% CI, 52%–86%) for H3N2 in a controlled trial comparing live and inactivated vaccines.[350] Vaccination of adults is also associated with decreased absenteeism from work or school and is significantly cost-effective,[29] but these benefits may not be seen in years when there is not a good match between vaccine and circulating viruses.[351]

A meta-analysis of eight randomized, controlled trials in healthy adults during 2004–2008 estimated the pooled efficacy of IIV against culture-confirmed influenza to be 59% (95% CI, 51–67) among those aged 18 through 64 years.[352] The role of antigenic mismatch in the efficacy observed in these trials is unclear, and some studies in young

adults have demonstrated high levels of efficacy (76%) despite a degree of antigenic mismatch. Recent studies using virus culture and/or PCR end points have demonstrated similar levels of efficacy for both egg-grown (78%) and cell culture–grown IIVs (84%).[353] The protective efficacy of the recombinant HA vaccine in healthy adults was approximately 47% in healthy adults in a study done in a single season with significant antigenic mismatch.[354]

Although annual vaccination of elders and other high-risk persons has been recommended for many years, there are few randomized trials demonstrating absolute efficacy in these groups, in part because the existing vaccine recommendations make it difficult to do studies using a placebo group. In the most commonly referenced study, IIV was 52% (95% CI, 29–67) efficacious in preventing serologically documented influenza illness in a population of adults 60 years of age and older.[270] When the groups were further stratified by age, efficacy estimates against serologically documented influenza illness were 57% (95% CI, 33%–72%) in those 60 through 69 years and 23% (95% CI, −51 to 61%) in those ≥70 years old. However, interpretation of this study is complicated by the use of postvaccination to postseason serologic response as definition of infection.

Use of higher doses of vaccine induces a stronger antibody response in older subjects, and in a large randomized study the relative efficacy of high-dose vaccine was 24% compared with standard-dose vaccine,[355] with reductions in small numbers of more severe end points, as well. As described earlier, rHA vaccine is also administered at a higher HA dose than standard-dose egg vaccine, and was also recently shown to have approximately 30% improved efficacy compared with standard-dose egg vaccine during a season with substantial H3N2 vaccine mismatch.[356] Standard-dose inactivated vaccine has been shown to be protective in limited studies in other high-risk groups, including those with HIV infection.[357,358]

Relatively few prospective trials have assessed inactivated vaccine efficacy in children. In one randomized, controlled trial in healthy children aged 6 through 23 months, vaccine efficacy was 66% (95% CI, 34–82) in the first year, but efficacy could not be assessed in the second year owing to a very low influenza attack rate.[359] In an early efficacy study comparing LAIV and IIV over 5 years in children, IIV3 had 77% efficacy against culture-confirmed H3 and 91% efficacy against H1.[360] In a large field trial conducted at multiple international sites in children 3 to 8 years old, IIV-4 was show to have efficacy of 59% in prevention of PCR-confirmed influenza, and 74% efficacy against moderate-to-severe influenza.[361]

Two large studies have evaluated the addition of MF59 to inactivated vaccine in children. In the first randomized placebo-controlled study done in children 6 to 72 months of age, the efficacy of IIV-3 against PCR-confirmed influenza was 43%, and that of the MF59 adjuvanted vaccine was 86%.[362] In a second study done in a population in which most children had previously been vaccinated, MF59 adjuvanted vaccine efficacy was similar to that of unadjuvanted vaccine in the entire study population, but was 31% better than standard vaccine in the subpopulation 6 to 23 months of age,[363] consistent with observations of greater adjuvant effect in immunologically naïve populations.

Intranasal Live Vaccines

LAIV was demonstrated to be efficacious in the prevention of influenza in a 2-year, randomized, placebo-controlled trial conducted in 1314 children 15 to 74 months of age. Efficacy against culture-confirmed influenza illness in the first year of this trial was 95% against influenza A/H3N2 and 91% against influenza B.[364] In the second year of the trial, the H3 component of the vaccine (A/Wuhan/93) was not a close match with the predominant H3 virus that season, A/Sydney/95. However, the efficacy of LAIV against this variant was 86% (95% CI, 75%–92%),[365] suggesting that LAIV can induce protective immunity against drift variants. Overall, the efficacy of LAIV to prevent any influenza illness during the 2-year period of surveillance in this field study was 92% (95% CI, 88%–94%). The overall efficacy of LAIV against culture-confirmed influenza among children 6 to <36 months who were attending daycare was shown to be 85% and 89% in the first and second years of the study, respectively.[366] Significant protection against flu-associated acute otitis media also was demonstrated (>90% in both years). Studies done in Asia have reached similar conclusions, with an efficacy of LAIV compared with placebo of between 64% and 84% over multiple seasons, depending on the antigenic match with the vaccine.[367]

Relatively few placebo-controlled trials of the efficacy of LAIV have been conducted in adults. In the human challenge model, LAIVs and IIVs were of approximately equal efficacy in prevention of experimentally induced influenza A (H1N1), A (H3N2), and B. The combined efficacy in preventing laboratory-documented influenza illness due to the three wild-type influenza strains was 85% for LAIV.[339] In a randomized, controlled study in healthy persons aged 1 through 64 years, of whom most of the participants were adults, the efficacy of a prelicensure, bivalent preparation of LAIV for preventing culture-confirmed influenza A illness in adults was 85% (95% CI, 70%–92%) for H1N1 and 58% (95% CI, 29%–75%) for H3N2.[350] LAIV was also evaluated in a large study against clinical end points performed in 4561 healthy working adults.[368] In this study, the effectiveness of LAIV in preventing severe febrile respiratory illness of any cause during the influenza season was 29%.

In a randomized, double-blind, placebo-controlled clinical trial of LAIV among community-dwelling ambulatory adults ≥65 years old, the overall efficacy of LAIV against viruses that were antigenically similar to the vaccine was 42%.[369] However, LAIV is not currently approved for use in adults older than 49.

Effectiveness (Results of Observational Studies)

Many recent studies have used a test-negative, case-control design, in which individuals meeting a particular case definition are tested for influenza with a highly sensitive and specific diagnostic test, and the vaccination exposure of test-positive cases and test-negative controls is determined.[370] Large surveillance networks for this purpose have been established in Canada, the United States, Europe, and Australia for purposes of making interim and end-of-season estimates of VE. Studies using this design have shown variable results, with estimates generally ranging from as low as 20%, or in some cases, no effectiveness, to as high as 60% to 70%. Although the various networks vary in their study design and the specific selection criteria for subject inclusion, a few overall generalizations can be stated. Failure to detect VE has typically occurred in studies with very low prevalence of influenza in the study population, or in years with substantial antigenic mismatch between the vaccine and circulating strains; but even in situations of antigenically matched viruses, VE remains in the 50% to 60% range.[371] In some cases, these viruses have been shown to have substantial changes on an HA sequence level despite appearing well matched according to traditional HAI tests.[372]

Most studies have not enrolled enough subjects in a single season to make age-specific estimates of VE. However, there is a trend toward decreased VE in elderly, not surprising given their diminished immune response to vaccination. After accumulating cases over several seasons, it is possible to use the same test-negative case-control design to demonstrate VE of approximately 60% against influenza-related hospitalization in a population of community dwelling older adults.[373] Among hospitalized patients, vaccinated individuals have lower rates of ICU admission and in-hospital deaths.[374]

Observational studies to compare different types of vaccine are dependent on market share, so complete information is not always available. In a cohort study based on Medicare beneficiary data in the United States, the use of high-dose vaccine was associated with a significantly greater reduction in influenza-related deaths among seniors in a year in which H3N2 viruses predominated, but not in a year of primarily pH1N1 virus.[375] In a case-control study among elderly individuals in Canada, MF-59 adjuvanted vaccine also appeared to be more effective than standard-dose unadjuvanted vaccine.[376]

Although the use of a study design in which testing is performed without knowledge of vaccination status may eliminate some biases related to health care access and health-seeking behavior, the results are influenced by the accuracy of the diagnostic testing, because errors in assignment to the case or control group will bias VE toward nil.[377] In a study done in children, it was demonstrated that with use of the test-negative case-control approach, estimates of VE were substantially

higher when children with documented infections with viruses other than influenza were used as a control group, rather than all children who were test-negative for influenza.[378]

Effects of Prior Vaccination

Studies of influenza vaccine effectiveness using the test-negative design, in addition to other study designs, have suggested that vaccine effectiveness is decreased in those who are yearly vaccine recipients, compared with those who receive vaccine for the first time in a given year. These findings would be consistent with very early observations made in a boys' boarding school, referred to as the "Hoskins effect." However, not all studies have shown a negative effect of prior vaccination. The effects seem to be greatest when the current season and the previous season vaccine components are similar to each other, but the circulating virus is a mismatch.[379]

The immunologic mechanisms that might be responsible for the negative effects of prior vaccination are not known. Individuals with higher prevaccination antibody levels tend to have lower-magnitude responses to vaccination, although still typically achieving very high levels of antibody. Prior vaccination might result in memory responses that preferentially recognize shared epitopes and not new epitopes that are present in antigenically drifted viruses, representing a form of back-boosting.[380]

Because at least one component of the vaccine changes each year, there is not a practical solution to influenza control using current vaccine strategies other than annual vaccination. Further research to understand the magnitude and mechanisms of putative negative effects of prior vaccination will clearly be important in improving influenza control.

Comparisons of Live and Inactivated Vaccines

Although relatively few randomized direct comparisons of the efficacy of live and inactivated vaccines have been performed, the available studies are consistent with the observed effects of age and prior influenza experience on immunogenicity. When these vaccines have been compared in young children 12 months through 59 months of age, LAIV has shown consistently superior protection, with an approximately 50% greater protective efficacy than inactivated vaccine.[294,381]

In contrast, studies that directly compared the vaccines in adults have suggested that the vaccines have similar efficacy, or that IIV vaccine is slightly more efficacious than live vaccine. In one three-armed study, the efficacy of LAIV compared with placebo for prevention of laboratory-confirmed influenza in healthy adults was 57%, whereas the efficacy of the IIV was 77%, but the difference between the two vaccines was not statistically significant.[382] In a subsequent season in the same population, the absolute efficacies of IIV and LAIV were 68% and 36%, respectively.[383] In an effectiveness assessment in the US military, the effectiveness of IIV against medical visits for pneumonia or other influenza related diagnoses was higher than that of LAIV, except for personnel who had not been vaccinated in previous years.[384]

When the H1N1 component of LAIV was replaced by a virus with the HA and NA of an A/California/09 virus after the pandemic, there was a substantial decrease in effectiveness of LAIV, particularly against H1N1.[385] This poor effectiveness was repeatedly demonstrated in the United States, and to a lesser extent in other countries. The reasons for the diminished effectiveness of this formulation are not completely known but may be related to the replication fitness of the specific H1N1 virus, increasing levels of background immunity in the vaccinated population, or other factors. However, as a result, the use of LAIV in the United States was not recommended by the Advisory Committee on Immunization Practices (ACIP) for several years. Subsequently, the H1N1 component has been updated with a virus (A/Slovenia) that appears to generate stronger immune responses, and use of LAIV has resumed. No information is available regarding the efficacy or effectiveness of this new formulation at this time.

Secondary Protection

In general, individuals at the highest risk for influenza complications may also be compromised in their ability to respond to vaccination. There has been considerable interest in potential strategies to protect such individuals indirectly by preventing illness and viral transmission within highly susceptible populations that probably play a role in community transmission, such as schoolchildren. Several studies have suggested that this may be possible. During the 1968 pandemic, it was observed that the incidence of respiratory illness during the period of influenza A circulation among unvaccinated adults was substantially lower in a community in which schoolchildren were immunized than in a comparison community with no school immunization. Influenza B was not contained in the vaccine, and during a subsequent influenza B epidemic there was no difference in adjusted respiratory illness rates in adults in the two communities.[386] In a recent study, closed agricultural communities of Hutterites were randomized to vaccination of schoolchildren with influenza vaccine, or with hepatitis A vaccine as a control. In the subsequent influenza A epidemic, the rate of laboratory-documented influenza A in unvaccinated adults residing in school-vaccinated communities was reduced by 61% (95% CI, 8%–83%) compared with adults in unvaccinated communities.[387] Vaccination of children in daycare has been reported to reduce the rates of febrile respiratory illnesses in unvaccinated household contacts.[388] Observations in Japan, where it appeared that substantial fluctuations in overall influenza-related mortality (occurring mostly in the elderly) were directly related to the rate of influenza vaccination in school-aged children, also support a potential role for school vaccination in protection of elders.[389] A retrospective study conducted in Japan demonstrated that universal vaccination of schoolchildren reduced the number of class cancellation days and absenteeism when compared with years during which the immunization program was abandoned.[390]

A critical target group for vaccination is health care workers.[391] At a minimum, universal vaccination of heath care workers will reduce workplace absences and prevent disruptions in care.[392] In addition, there is supportive evidence that vaccination of health care workers reduces the risk of nosocomial influenza in hospitals.[393] In nursing homes, vaccination of staff reduces mortality in residents independently of the vaccination status of the residents themselves.[394,395]

Maternal Immunization

Infants younger than 6 months are at substantial risk for influenza-related morbidity but are too young to receive influenza vaccine. One strategy to protect vulnerable infants is maternal immunization, with protection mediated both by transfer of maternal antibody as by reduced potential for contact with an influenza-infected mother. In multiple randomized studies of maternal immunization, infants born to mothers immunized with influenza vaccine have had substantially lower rates of laboratory-documented influenza in the first 6 months of life than did infants born to mothers immunized with control vaccine.[396–398] Similarly, in a retrospective case-control study, the frequency of influenza immunization was substantially lower in the mothers of infants hospitalized with PCR-confirmed influenza than in mothers of hospitalized infants who were PCR negative, with an estimated protective effect of 92%.[399]

Recommendations for Vaccine Use

Current US recommendations are for routine annual influenza vaccination of all individuals aged 6 months and older. Ideally, vaccine should be administered by October, but later vaccination can still be beneficial if the seasonal epidemic has not yet occurred. Recommendations for influenza vaccine in the United States are updated annually by the CDC. (Also see Chapter 316.)

Vaccines for Pandemic Influenza

As described earlier, there have been multiple observations of human infection with avian or swine influenza viruses with novel HA and/or NA subtypes, and there continues to be concern regarding the potential of such viruses to acquire the ability to transmit efficiently from person to person and result in the next human influenza pandemic. In addition to active ongoing surveillance, there have been substantial efforts to prepare for such a pandemic by developing potential pandemic vaccines in advance. Because seasonal vaccines are licensed for use in all age groups and have substantial evidence of safety and efficacy, pandemic preparedness efforts have generally focused on development of pandemic formulations of seasonal vaccines, including inactivated and live varieties. Several of these have undergone advanced clinical development and

have been licensed or approved for emergency use based on a favorable safety and immunogenicity profile.

A large number of inactivated H5 and H7 vaccines have been developed and tested in humans. These vaccines have included egg-derived, cell culture–grown, and recombinant HA vaccines similar to the approaches taken for seasonal influenza. Extensive clinical trials have generally shown that these vaccines are well tolerated, but when administered without an adjuvant, poorly immunogenic. Generation of potentially protective immunity, as measured by serum HAI and neutralizing antibody responses, requires a two-dose schedule and the use of doses substantially higher than those used for seasonal vaccines.[400–402] When responses are seen, they are typically limited in breadth and predominantly focused on the homologous antigen.[403] Limited studies of whole-virion vaccines have suggested slight improvements in immunogenicity.[404]

Oil-in-water emulsions such as MF59, AS03, and stable emulsion (SE) have allowed much lower doses of pandemic vaccines to be used successfully.[405,406] With these adjuvants, substantial immune responses have been seen at doses as low as 3.75 μg of antigen, compared with the need to use 45 μg or 90 μg of unadjuvanted vaccine, allowing for the generation of a much larger supply of vaccine in the event of a pandemic. In addition, responses after adjuvanted vaccine are broader, with higher titers of antibody recognizing antigenic variants of the vaccine subtype.[407,408] A number of other adjuvants have also been tested with pandemic vaccines and also show effective improvement in response. Limited studies have suggested that the use of an adjuvant is much more important with the first dose of vaccine.[409] However, even with adjuvants, inactivated pandemic vaccination will likely require a two-dose schedule, imposing significant operational challenges to a pandemic response.

Despite the relatively poor immunogenicity of these vaccines, multiple studies have shown that even unadjuvanted pandemic vaccines may induced long-lasting immune memory, demonstrated by vigorous responses to subsequent booster doses many years later.[410,411] Such responses can be demonstrated even in subjects who did not respond to the primary series. Markers of immune priming have not been identified in these studies.

Live-attenuated pandemic vaccines have also been evaluated in clinical trials. Evaluation of pandemic live vaccines is complicated by the concerns regarding potential transmission of the vaccine virus, possible reassortment with cocirculating human influenza viruses, and potential generation of virulent human viruses with novel HA or NA surface proteins. Therefore, these studies have been conducted under isolation conditions to prevent possible transmission outside the study, and consequently have been somewhat limited in size.

Surprisingly, all of these studies have shown very limited replication of the pandemic vaccine viruses on either the A/Ann Arbor or the A/Leningrad backbone.[412,413] Vaccine virus shedding occurs in a minority of susceptible recipients, and when it is detected, is almost invariably of limited magnitude and short duration. Because immune correlates of protection afforded by seasonal live vaccines have not been determined, it is not possible to draw definitive conclusions regarding the relevant immunogenicity of pandemic formulations of these vaccines. However, measurable serum or mucosal immune responses occur rarely.

Similar to the findings with inactivated vaccines, pandemic formulations of LAIV appear to induce long-lasting immune memory despite the lack of measureable immune response to the initial immunization.[414–416] These observations have caused some to suggest that the most effective use of vaccines for pandemic control would be prepandemic vaccination,[417] in which the population would be vaccinated with a prototypic H5, H7, or other threat subtype prior to a pandemic, and then boosted with an antigenically matched virus should a pandemic occur.

Strategies for More Broadly Protective Vaccines

For a variety of reasons previously outlined, there is considerable interest in the development of influenza vaccine approaches that could generate more broadly protective immunity, potentially providing protection against multiple antigenic variants within a subtype, or possibly even providing protection against multiple subtypes. Several approaches have emerged in pursuit of this goal.

In contrast to the globular head of the HA, the stalk, or HA2 region, remains relatively conserved from season to season. Stalk-directed antibody or stalk-reactive B cells are seen most frequently when individuals are exposed to a novel influenza virus, such as after infection with H5N1[214] or pH1N1 viruses in 2009.[215] Stalk-specific antibodies are capable of mediating virus neutralization by inhibiting HA mediated fusion.[418] In addition, these antibodies can mediate antibody-dependent cellular cytotoxicity. Therefore development of vaccines to induce stalk antibodies is one of the prime strategies for more broadly protective vaccines.[218] Both chimeric approaches, wherein several doses of a vaccine in which the HA1 domain is derived from a novel subtype and the stalk remains the same,[419,420] and stalk-only constructs[421–423] are in development.

The influenza NA has also been explored as a potential target for more broadly protective vaccines. The rate at which mutations accumulate in the NA appears to be less than that in the HA, [424] suggesting that vaccines that induced substantial NA specific immunity would continue to provide protection against drifted viruses, and would need updating less often than HA-centric vaccines.

The third envelope protein, M2, has also been identified as a potential target because the extracellular domain (M2e) is fairly well conserved among human influenza A viruses. The mechanism of protection by M2e antibodies also involves antibody-dependent cellular cytotoxicity in M2e antibody-mediated protection.[227] Multiple platforms have been used to induce antibody specific for M2e, which have resulted in variable levels of M2e antibodies in humans.[425]

Vaccines designed primarily to elicit cellular antibodies often contain mixtures of peptides computationally identified as cellular target epitopes. Immunization of humans with a mixture of linear peptides has been reported to induce cellular immunity and prime for subsequent vaccine responses.[426] Such epitopes could be delivered by mixtures of peptides, or by live viral vectors, such as vaccinia.[427,428] Virus-like particles,[429] in addition to gamma-irradiated whole virus,[430] have also been evaluated for their ability to induce cellular immune responses.

ANTIVIRAL AGENTS (Also See Chapter 45)

Five antiviral drugs in two classes are currently available for the prevention and treatment of influenza, and several more are in the advanced stages of development. A comparison of the basic pharmacology and antiviral activity of the available agents is given in Table 165.8, and they are described in detail later. It is important to recognize that individuals with an intact immune system who have had previous influenza infections rapidly limit the replication of these viruses. Therefore the opportunity to affect viral replication with antiviral agents is limited, and effective use of these agents requires early initiation of therapy.

M2 Inhibitors: Amantadine and Rimantadine

Mechanism of Action and Activity

Currently, all circulating influenza viruses are resistant to the M2 inhibitors amantadine and rimantadine. However, they are described briefly because changes in susceptibility are unpredictable and they may become useful in the future. The M2 inhibitors amantadine and rimantadine are related primary symmetrical amines and are active against influenza A virus, but not influenza B or C virus, in a variety of cell culture systems and animal models. The antiviral activity of these drugs is the result of inhibition of the M2 ion channel activity of susceptible viruses. The function of the M2 ion channel in viral replication is to acidify the interior of the virion, allowing the ribonucleoproteins to be transported to the nucleus.[431] Thus, the antiviral effect primarily manifests in cell culture as inhibition of virus uncoating. Currently available M2 inhibitors are active against only influenza A virus.

Pharmacology and Side Effects

Although the mechanism of action and spectrum of activity for amantadine are similar to those for rimantadine, there are important pharmacokinetic differences between the two drugs. Amantadine does not undergo metabolic change and is excreted unchanged in the urine

TABLE 165.8 Antiviral Agents for Influenza

	AMANTADINE[a]	RIMANTADINE[a]	ZANAMIVIR	OSELTAMIVIR	PERAMIVIR	BALOXAVIR
Protein target	M2	M2	Neuraminidase	Neuraminidase	Neuraminidase	Cap-dependent endonuclease
Activity	A only	A only	A and B	A and B	A and B	A and B
Side effects	CNS (13%) GI (3%)	GI (6%) GI (3%)	Bronchospasm	GI (9%)	GI (8%)	Diarrhea, bronchitis
Metabolism	None	Multiple (hepatic)	None	Hepatic	None	UGT1A3, CYP3A4
Excretion	Renal	Renal + others	Renal	Renal (tubular secretion)	Renal	Fecal (80%): urine (15%)
Drug interactions	Antihistamines, anticholinergics	None	None	Probenecid (increased levels of oseltamivir)	—	Antacids and laxatives reduce concentration
Dose adjustments needed	≥65 yr old CrCl <50 mL/min	≥65 yr old CrCl <10 mL/min	None	CrCl <30 mL/min Severe liver dysfunction	CrCl <30 mL/min	—
Contraindications	Acute-angle glaucoma	Severe liver dysfunction	Underlying airway disease			—
FDA-Approved Indications						
Therapy	Adults and children aged ≥1 yr	Adults only	Adults and children aged ≥7 yr	Adults and children aged ≥2 wk	Adults who cannot take oral or inhaled medications	Adults and children ≥12 yr of age[b]
Prophylaxis	Yes	Yes	Adults and children aged ≥5 yr	Adults and children aged ≥1 yr	—	—

[a]Currently circulating influenza viruses are uniformly resistant to amantadine and rimantadine.
[b]Approved for acute, uncomplicated influenza of ≤48-hr duration.
CNS, Central nervous system; *CrCl,* creatinine clearance; *FDA,* US Food and Drug Administration; *GI,* gastrointestinal.

with a half-life of 12 to 18 hours. Rimantadine undergoes extensive metabolism, and less than 15% of the drug is excreted in the urine unchanged.

The most common side effects of amantadine are minor and reversible central nervous system (CNS) side effects such as insomnia, dizziness, and difficulty in concentrating. These side effects may be more troublesome in older adults. Rimantadine is associated with less CNS effect, and in comparative studies of long-term administration the rate of CNS side effects was not significantly different from the rate with placebo.[432]

Efficacy

Both amantadine and rimantadine are effective in the therapy of experimentally induced and naturally occurring influenza A. Amantadine treatment within the first 48 hours of illness is associated with decreases in the duration of fever and more rapid resolution of clinical symptoms, and more rapid improvement in small airway dysfunction in healthy adults with uncomplicated influenza. Studies of rimantadine therapy of acute influenza in otherwise healthy adults with uncomplicated influenza have shown levels of benefit essentially identical to those seen with amantadine.[433,434] Rimantadine has also been evaluated in the treatment of influenza A in children, with variable effects on clinical symptom scores; one study showed a decrease in scores and fever compared with acetaminophen,[435] and the other showed no significant difference.[436]

Drug Resistance

Drug resistance viruses emerge frequently in treated individuals, particularly children, in whom subpopulations of resistant virus can be detected after treatment in virtually all cases.[437] Resistance is the result of single point mutations in the membrane-spanning region of the M2 protein, and it confers complete cross-resistance between amantadine and rimantadine.[438] Resistant virus can be transmitted to, and can cause disease in, susceptible contacts. Prolonged shedding of resistant viruses may occur in immunocompromised patients, particularly children, and may continue even after therapy is terminated,[439] consistent with the relative fitness of these resistant viruses. Although previously rare, a rapid increase in the prevalence of de novo resistance to M2 inhibitors was noted in 2005,[440,441] and the emerging pH1N1 viruses are also completely resistant to the M2 inhibitors.

Neuraminidase Inhibitors: Zanamivir, Oseltamivir, and Peramivir

Mechanism of Action and Activity

The NIs act by inhibiting the functioning of the influenza virus NA. This enzyme cleaves terminal sialic acid from sialic acid–containing glycoproteins that serve as host cell receptors for attachment of influenza viruses. As virus replication proceeds within the cell, NA is synthesized and transported to the cell surface, where it removes the sialic acid from these cell surface glycoproteins. Destruction of these receptors by NA is critical in allowing newly formed viruses to subsequently egress from the cell and spread to other cells. Studies with mutant, NA-deficient viruses have shown that in the absence of a functional NA, virus remains attached to the host cell and to other virions.[442] In addition, NA may be important in facilitating the penetration of virus through secretions in the respiratory tract, which are rich in sialic acid–containing macromolecules.[443]

NIs are active against influenza viruses at millimolar concentrations or less. Activity against clinical isolates assessed in plaque inhibition tests ranges from concentrations of 0.01 to 16 μM. Influenza B viruses are approximately 10-fold less sensitive than influenza A viruses, but they are still sensitive well within clinically achievable concentrations. Among the influenza viruses sensitive to NIs are avian viruses with all nine known NA subtypes.

Pharmacology and Side Effects

Although all three drugs have identical mechanisms of action and similar profiles of antiviral activity, they have different pharmacologic properties. Oseltamivir is rapidly absorbed from the gastrointestinal tract and is converted in the liver by hepatic esterases to the active metabolite, oseltamivir carboxylate. The metabolite is excreted unchanged in the urine by means of tubular secretion, with a serum half-life of 6 to 10 hours. Administration of the drug with food may improve tolerability without affecting drug levels. Zanamivir is not bioavailable by the oral route and must be administered topically to be effective. The drug is supplied in blister packs in which each blister contains 5 mg of zanamivir and 20 mg of lactose carrier. The standard dose is therefore two inhalations twice a day. Peramivir was initially studied as an orally active agent, but pharmacodynamic evaluation determined that oral absorption was not sufficient for this mode of administration.[444] The drug is now

licensed as an intravenous, long-acting formulation that can be administered as a single dose.[445]

The major adverse effects reported for oseltamivir have been gastrointestinal upset, probably irritation due to rapid release of the drug in the stomach. Rates of nausea can be substantially reduced if the drug is taken with food. The most commonly reported adverse effects in individuals treated with zanamivir have been diarrhea, nausea, and nasal signs and symptoms, which have occurred at essentially the same rate as in placebo recipients. Peramivir has a similar safety profile in studies conducted to date. In one study in which zanamivir was used in influenza-infected patients with asthma or chronic obstructive pulmonary disease, the frequency of significant changes in FEV_1 or peak flow rates was higher in zanamivir than in placebo recipients. For this reason, individuals with these pulmonary conditions should have ready access to a rapidly acting bronchodilator when using zanamivir, in the event that the drug precipitates bronchospasm.

The dose of oseltamivir should be reduced to 75 mg once daily in individuals with renal impairment (i.e., with creatinine clearance of less than 30 mL/min). No data are available regarding the use of the drug in individuals with more significant levels of renal impairment. Likewise, no information is available regarding the use of oseltamivir in individuals with hepatic impairment. Clinically significant drug interactions have not been reported. Because oseltamivir is eliminated by means of tubular secretion, probenecid increases serum levels of the active metabolite approximately twofold. However, dosage adjustments are not necessary in individuals taking probenecid. Dosage reductions are also recommended in renal impairment for peramivir.

Although significant increases in the serum half-life of zanamivir are seen in the presence of renal failure, the small amounts of the drug that are absorbed systemically suggest that dosage adjustments would not be necessary. Studies of the pharmacokinetics of the drug in the presence of impaired hepatic function have not been reported.

Efficacy

In studies of naturally occurring, uncomplicated influenza in healthy adults, therapy with oseltamivir initiated within the first 36 hours of symptoms resulted in 30% to 40% reductions in the duration of symptoms and severity of illness and reduced rates of prolonged coughing.[446,447] In addition, early therapy is associated with a significantly earlier return to work or other normal activities. Similarly, in healthy adults, early therapy of uncomplicated influenza A or B with inhaled zanamivir has been shown to result in a reduction of approximately 0.8 to 1.5 days in the duration of influenza symptoms, and an earlier return to normal activities.[448,449] A study of a single dose of intravenous peramivir in uncomplicated influenza in otherwise healthy adults demonstrated an approximately 21-hour decrease in the duration of symptoms[450] However, in a study of hospitalized adults, peramivir offered no benefit over standard of care.[451]

Both oseltamivir and zanamivir have been evaluated as therapy for children. Administration of oseltamivir liquid at a dose of 2 mg/kg per dose twice daily for 5 days was well tolerated and resulted in a 36-hour reduction in the duration of symptoms in children with influenza A.[452] In addition, the use of oseltamivir was associated with a 44% reduction in the frequency of otitis media complicating influenza, and with reductions in antibiotic prescriptions in influenza-infected children. Similarly, therapy of children 5 to 12 years old with symptomatic influenza A and B virus infection who were treated within 36 hours with inhaled zanamivir (10 mg twice a day) resulted in relief of symptoms 1.25 days earlier than in placebo recipients, and a more rapid return to normal activities.[453]

NI therapy of influenza in adults with risk factors for influenza complications has not been evaluated extensively. However, both drugs have shown trends toward efficacy in such populations.[449,454] The results of meta-analyses of the pooled data from phase III studies have indicated that early treatment with inhaled zanamivir is associated with a median reduction of illness of 2.5 days in older adult and high-risk subjects, and a 3-day earlier return to normal activities.[455]

Initial placebo-controlled trials of NI therapy conducted primarily in otherwise healthy adults did not capture substantial numbers of complications. However, pooled analyses of these studies of early therapy with zanamivir[455] and oseltamivir[456,457] demonstrated a significant reduction in the rate of influenza complications in treated individuals. The subsequent experience in the emerging epidemic of pH1N1 virus has also suggested a beneficial effect of early therapy on complications. These include observations in hospitalized patients[458,459] and surveillance data suggesting that therapy as late as 5 days improved survival of hospitalized patients.[460] Surveillance data have also suggested that treated children had lower rates of complications.[461]

Drug Resistance

Analysis of drug-resistant viruses has revealed two basic mechanisms of resistance that illustrate the interactive roles of the viral HA and NA in binding to and release from infected cells. Mutations within the catalytic framework of the NA that abolish binding of the drugs are the most common drug resistance mutations.[462,463] The mutations conferring resistance are dependent on the specific NA; that is, common resistance mutations in N1 (e.g., H274Y) are different from the ones seen in the N2 (e.g., R292K or E119V) or influenza B (e.g., D198N). In addition, depending on the mutation, these viruses may be specifically resistant to only one inhibitor.[464]

A second type of mutation associated with cell-cultured resistant viruses involves mutations in the receptor binding region of the hemagglutinin. HA mutations associated with resistance to NIs reduce the affinity of the HA for its receptor, allowing cell-to-cell spread of virus in the absence of NA activity.[462,465] Inhibitor resistance due to HA mutations has not been identified as a clinically significant issue to date, but does illustrate the importance of balance between HA and NA activities in viral replication.

It is even possible to generate inhibitor-dependent viruses, in which the affinity for the receptor is apparently so low that NA activity must be inhibited to allow the virus to bind at all. Resistant viruses with HA mutations exhibit cross-resistance to these drugs in cell culture but may retain susceptibility in animal models. Many of these viruses also exhibit reduced virulence in animals.

Drug-resistant viruses were isolated infrequently from oseltamivir-treated individuals in clinical trials, being seen in less than 2% of treated adults and detected in 5.6% of children.[452] Subsequently, it was demonstrated that resistant viruses could be detected in up to 18% of treated children with use of sensitive PCR techniques to pick up minor subpopulations.[466] However, development of resistance has not generally been associated with more severe illness or failure of therapy. Antiviral therapy in highly immunocompromised individuals who may have difficulty clearing virus is frequently associated with development of resistance.

Some resistant viruses appear to have reduced fitness, with reduced levels of replication, attenuation in animals, and reduced ability to be transmitted from animal to animal.[467–470] Therefore, there had been hope that resistance would not be the same limiting issue with NA inhibitors as it has been with the M2 inhibitors. However, other resistance mutations, particularly the H274Y mutation, do not affect the fitness of the virus or transmission in animal models.[471] Beginning in 2006, spontaneously resistant H1N1 viruses carrying the H274Y mutation began to be detected in viruses from individuals who did not have a history of exposure to oseltamivir.[472] By 2008, all H1N1 viruses isolated in the United States were resistant to oseltamivir.

The emerging pH1N1 viruses, which replaced previously circulating H1N1 viruses, have largely remained sensitive to NIs to date.[473] Localized outbreaks of pH1N1 viruses with the H274Y resistance mutation have been reported[474] and these viruses clearly have the capacity to spread and become dominant just as their predecessor seasonal H1N1 viruses did. However, current worldwide surveillance indicates that the majority of seasonal influenza A and B viruses remain sensitive to NIs.

Antiviral Agents in Development

The novel antiviral drug pimodivir is a nonnucleoside inhibitor of the endonuclease cap-stealing function of the PB2 polymerase, and is therefore active against viruses resistant to adamantanes or NIs. However,

this drug is active against only influenza A viruses. In the challenge model, administration of pimodivir for 5 days beginning 24 hours after challenge with A/Wisconsin/05 (H3N2) virus resulted in reduced viral shedding and clinical symptom scores.[475] In a subsequent trial in uncomplicated influenza, pimodivir reduced viral shedding compared with placebo but did not reduce symptoms.[476] Baloxavir marboxil (Xofluza) is a cap-dependent endonuclease inhibitor of influenza A and B viruses. It has been approved in Japan (December 2017) and the United States (October 2018) as a single-dose treatment for uncomplicated influenza in adults and children ≥12 years of age.[476a] Controlled trials of baloxavir in naturally occurring influenza A (H3N2) infection in outpatients showed alleviation of symptoms and a reduction in viral shedding within 24 hours of administration.[476b] (Also see Chapter 45.)

Other agents in development include the long-acting topically applied NI laninamivir,[477] the broadly acting polymerase inhibitor favipiravir,[478] and nitazoxanide,[479] an inhibitor of HA maturation that also affects the host response. (Also see Chapter 45.)

The combination of amantadine, ribavirin, and oseltamivir has been assessed in the treatment of influenza based on encouraging preclinical results suggesting synergistic effects even against viruses resistant to amantadine.[480] Uncontrolled observations in humans had also suggested potentially improved effectiveness compared with oseltamivir alone.[481] However, a large prospective randomized trial showed no clinical benefit of combined therapy compared with oseltamivir monotherapy, although there was slightly faster viral clearance in the group receiving combined therapy.[482] Because of concerns regarding development of resistance, combination therapy may become a very important approach as additional effective antiviral agents with new mechanisms of action become available.

Recommendations for Therapeutic Use of Antivirals

There are several general features that guide the use of antivirals for influenza. First, the bulk of viral replication in persons with normal immune systems takes place very early in the course of illness. Not surprisingly, repeated studies have demonstrated that the effectiveness of therapy primarily depends on the rapidity with which treatment is initiated. However, in persons with severe illness or prolonged viral replication, even late therapy can occasionally be of benefit. Second, most healthy persons with uncomplicated influenza will recover completely without therapy. Thus, therapy is not necessarily universally indicated in all persons with influenza, but can be lifesaving in some persons. Third, resistance to antivirals has been observed frequently with almost all influenza antivirals, and the potential for generation of resistant viruses is an important component of therapy recommendations.

Guidelines for the therapy of influenza have been published[261] and are frequently updated as new information regarding susceptibility and the availability of new agents becomes available. Recommendations are also available from multiple online sources, particularly the CDC influenza website (http://www.cdc.gov/flu). In general, therapy should be started as soon as possible, and should be primarily used in individuals with severe influenza or who have a higher risk for influenza complications (see Table 165.6). Diagnostic tests for influenza, particularly nucleic acid tests such as PCR assays, are increasing in availability and sensitivity. However, in select situations, treatment based on a high degree of clinical suspicion may be appropriate.

There is general agreement that persons who are hospitalized with laboratory-documented influenza, or in whom the degree of clinical suspicion of influenza is high, should receive antiviral therapy. Although the greatest benefit is seen when therapy is initiated within 48 hours of symptom onset, the experience during the H1N1 pandemic supports the benefit of treatment of individuals who remain influenza positive, even if therapy is initiated later in the course of illness.[460,483] Therapy should also be administered to individuals with laboratory-confirmed or highly suspected influenza who are at high risk of influenza complications, if therapy can be initiated within 48 hours. The benefits of therapy in high-risk patients after 48 hours are not clear, but antiviral therapy should be strongly considered if the illness is progressing, particularly if they remain influenza positive.

Therapy can also be considered in otherwise healthy persons with laboratory-documented or highly suspected influenza who present within 48 hours of the onset of symptoms, and who wish to reduce the duration of symptoms. However, the decisions regarding treatment should consider the relatively small benefit demonstrated in clinical trials and the overall low risk of complications. The benefits of later therapy in otherwise uncomplicated influenza are unclear.

Decisions regarding the specific antiviral agent will depend greatly on the most current information regarding the resistance patterns of circulating influenza viruses. At the current time, almost all seasonal influenza A and B viruses would be expected to be resistant to the M2 inhibitors and sensitive to the NIs oseltamivir and zanamivir.

Chemoprophylaxis

Several schemes for prophylaxis have been evaluated, including seasonal prophylaxis, wherein drug is administered throughout the influenza epidemic season, generally 4 to 6 weeks; family prophylaxis, wherein drug is administered to family members for a short period of time after recognition of an index case in the family; and outbreak-initiated prophylaxis in institutions, which could be considered to be a variation on the theme of family prophylaxis. In addition, short-term antiviral prophylaxis can be considered for high-risk individuals who are vaccinated during the influenza season.

Seasonal Prophylaxis

Seasonal prophylaxis with amantadine and rimantadine has been shown to provide similar levels of protection against influenza A, ranging from 70% to 90%[432] Both zanamivir and oseltamivir have also been shown to be protective in seasonal prophylaxis. In healthy adults, inhaled zanamivir was shown to have about 67% efficacy for prevention of confirmed influenza,[484] and in a similar study the efficacy of oral oseltamivir was 74%.[485] Both drugs were well tolerated on prolonged use.

Relatively less information is available about the use of any of the influenza antivirals for prophylaxis in older adult or high-risk populations. In one study, seasonal prophylaxis was highly effective in preventing laboratory-documented influenza in older adult residents of retirement communities.[486] Important to note, 80% of the subjects had previously been vaccinated, and prophylaxis resulted in a 91% reduction in influenza in this group. Thus, vaccine and chemoprophylaxis had an additive protective effect in older adults.

Family Prophylaxis

Results of outbreak prophylaxis in the family setting with amantadine or rimantadine have depended on whether both the index case and the contacts are treated. When the index case was not treated with amantadine, protection of family contacts has been seen, but if the index case was treated with amantadine at the same time that contacts received prophylaxis, no protection was seen, because of the rapid generation and transmission of resistant virus. This problem has not been seen in studies of family prophylaxis with oseltamivir[487] or zanamivir.[488] In general, drug is administered to contacts for 5 to 7 days after recognition of the index case. It is important to realize that treated individuals remain susceptible to infection from outside the family after such prophylaxis is discontinued.

Outbreak Prophylaxis

Probably one of the most common uses of antiviral agents for influenza is to terminate the transmission of influenza within institutions such as nursing homes during outbreaks. Both adamantanes and NIs have been used to control outbreaks in institutional settings, combined with other infection control practices. Prophylaxis should begin as early as possible within the institution, and continue for 7 to 10 days.[261] If possible, individuals who are receiving treatment should be isolated from those who are receiving prophylaxis to reduce the likelihood of generation and transmission of resistant viruses within the institution.[489,490]

Key References

The complete reference list is available online at Expert Consult.

3. Smith W, Andrewes CH, Laidlaw PP. A virus obtained from influenza patients. *Lancet*. 1933;2:66–68.
24. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA*. 2003;289:179–186.
36. Siston AM, Rasmussen SA, Honein MA, et al. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. *JAMA*. 2010;303:1517–1525.
41. Louie JK, Acosta M, Samuel MC, et al. A novel risk factor for a novel virus: obesity and 2009 pandemic influenza A (H1N1). *Clin Infect Dis*. 2011;52:301–312.
47. Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. *N Engl J Med*. 2006;355:31–40.
48. Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974-1976. *N Engl J Med*. 1978;298:587–593.
63. Moser MR, Bender TR, Margolis HS, et al. An outbreak of influenza aboard a commercial airliner. *J Epidemiol*. 1979;110:1–6.
79. Kilbourne ED. *Influenza*. New York: Plenum Publishing; 1987.
81. Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. *J Virol*. 1989;63:4603–4608.
106. Gostic KM, Ambrose M, Worobey M, et al. Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. *Science*. 2016;354:722–726.
108. Burke DF, Trock SC. Use of influenza risk assessment tool for pandemic preparedness. *Emerg Infect Dis*. 2018;24:471–477.
132. Schwarzmann SW, Adler JL, Sullivan RFJ, et al. Bacterial pneumonia during the Hong Kong influenza epidemic of 1968-1969. *Arch Intern Med*. 1971;127:1037–1041.
153. Kwong JC, Schwartz KL, Campitelli MA, et al. Acute myocardial infarction after laboratory-confirmed influenza infection. *N Engl J Med*. 2018;378:345–353.
171. Hayden FG, Fritz R, Lobo MC, et al. Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. *J Clin Invest*. 1998;101:643–649.
207. Epstein SL. Prior H1N1 influenza infection and susceptibility of Cleveland family study participants during the H2N2 pandemic of 1957: and experiment of nature. *J Infect Dis*. 2006;193:49–53.
212. Black S, Nicolay U, Mesikari T, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. *Pediatr Infect Dis J*. 2011;30:1081–1085.
216. Ekiert DC, Bhabha G, Elsliger M-A, et al. Antibody recognition of a highly conserved influenza virus epitope. *Science*. 2009;324:246–251.
221. Schulman JL, Khakpour M, Kilbourne ED. Protective effects of hemagglutinin and neuraminidase antigens on influenza virus: distinctiveness of hemagglutinin antigens of Hong Kong - 68 virus. *J Virol*. 1968;2:778.
223. Clements ML, Betts RF, Tierney EL, et al. Resistance of adults to challenge with influenza A wild-type virus after receiving live or inactivated virus vaccine. *J Clin Microbiol*. 1986;23:73–76.
243. Forrest BD, Pride MW, Dunning AJ, et al. Correlation of cellular immune responses with protection against culture-confirmed influenza virus in young children. *Clin Vaccine Immunol*. 2008;15:1042–1053.
244. Sridhar S, Begom S, Bermingham A, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. *Nat Med*. 2013;19:1305–1312.
261. Harper SA, Bradley JS, Englund JA, et al. Seasonal influenza in adults and children—diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the infectious diseases society of America. *Clin Infect Dis*. 2009;48:1003–1032.
267. Zost SJ, Parkhouse K, Gumina ME, et al. Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains. *Proc Natl Acad Sci USA*. 2017;114:12578–12583.
273. Grohskopf LA, Sokolow LZ, Broder KR, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the advisory committee on immunization practices - United States, 2017-18 influenza season. *MMWR Recomm Rep*. 2017;66:1–20.
314. Nayak JL, Fitzgerald T, Richards KA, et al. CD4 T-cell expansion predicts neutralizing antibody responses to monovalent inactivated pandemic H1N1 influenza vaccine. *J Infect Dis*. 2013;207:297–305.
346. Nakaya HI, Wrammert J, Lee EK, et al. Systems biology of vaccination for seasonal influenza in humans. *Nat Immunol*. 2011;12:786–795.
352. Osterholm MT, Kelley NS, Sommer A, et al. Influenza vaccine efficacy and effectiveness: a new look at the evidence. *Lancet Infect Dis*. 2012;12:36–44.
355. DiazGranados CA, Dunning AJ, Kimmel M, et al. Efficacy of high-dose versus standard-dose influenza vaccine in older adults. *N Engl J Med*. 2014;371:635–645.
356. Dunkle LM, Izikson R, Patriarca P, et al. Efficacy of recombinant influenza vaccine in adults 50 years of age or older. *N Engl J Med*. 2017;376:2427–2436.
361. Jain VK, Rivera L, Zaman K, et al. Vaccine for prevention of mild and moderate-to-severe influenza in children. *N Engl J Med*. 2013;369:2481–2491.
364. Belshe RB, Mendelman PM, Treanor J, et al. The efficacy of live attenuated cold-adapted trivalent, intranasal influenza virus vaccine in children. *N Engl J Med*. 1998;358:1405–1412.
370. Orenstein EW, de Serres G, Haber MJ, et al. Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. *Int J Epidemiol*. 2007;36:623–631.
379. Skowronski DM, Chambers C, Sabaiduc S, et al. A perfect storm: impact of genomic variation and serial vaccination on low influenza vaccine effectiveness during the 2014–2015 Season. *Clin Infect Dis*. 2016;63:21–32.
446. Treanor JJ, Hayden FG, Vrooman PS, et al. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized, controlled trial. *J Am Med Assoc*. 2000;283:1016–1024.
448. Hayden FG, Osterhaus ADME, Treanor JJ, et al. Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infections. *N Engl J Med*. 1997;337:874–880.
450. Kohno S, Kida H, Mizuguchi M, et al. Efficacy and safety of intravenous peramivir for treatment of seasonal influenza virus infection. *Antimicrob Agents Chemother*. 2010;54:4568–4574.
460. Louie JK, Yang S, Acosta M, et al. Treatment with neuraminidase inhibitors for critically ill patients with influenza A (H1N1)pdm09. *Clin Infect Dis*. 2012;55:1198–1204.
464. Moscona A. Oseltamivir resistance - disabling our influenza defenses. *N Engl J Med*. 2005;353:2633–2636.

References

1. Hirsch A. *Handbook of Geographical and Historical Pathology*. 2nd ed. London: New Syndenham Society; 1883.
2. Crosby AW. *Epidemic and Peace*. Vol. part IV. Westport, CT: Greenwood Press; 1918:1976.
3. Smith W, Andrewes CH, Laidlaw PP. A virus obtained from influenza patients. *Lancet*. 1933;2:66–68.
4. Francis T Jr. A new type of virus from epidemic influenza. *Science*. 1940;92:405–408.
5. Taylor RM. A further note on 1233 ("influenza C") virus. *Arch Gesamte Virusforsch*. 1951;4:485–495.
6. Burnet FM. Influenza virus on the developing egg I. changes associated with the development of an egg-passage strain of virus. *Br J Exp Pathol*. 1936;17:282–295.
7. Mogabgab WJ, Green IJ, Dierkhising OC. Primary isolation and propagation of influenza virus in cultures of human embryonic renal tissue. *Science*. 1954;120:320–321.
8. Hirst GK. The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus. *Science*. 1941;94:22–23.
9. Francis T Jr, Salk JE, Pearson HE, et al. Protective effect of vaccination against influenza A. *Proc Soc Exp Biol Med*. 1944;55:104–105.
10. Smorodintseff AA, Tushinsky KMD, Drobyshevskaya AI, et al. Investigation of volunteers infected with the influenza virus. *Am J Med Sci*. 1937;194:159–170.
11. Kido H, Yokogoshi Y, Sakai K, et al. Isolation and characterization of a novel trypsin-like protease found in rat bronchiolar Clara cells: a possible activator of the viral fusion glycoprotein. *J Biolog Chem*. 1992;267:13573–13579.
12. Kawaoka Y, Webster RG. Sequence requirements for cleavage activation of influenza virus hemagglutinin expressed in mammalian cells. *Proc Natl Acad Sci U S A*. 1988;85:324–328.
13. Stieneke-Grober A, Vey M, Angliker H, et al. Influenza virus hemagglutinin with multibasic cleavage site is activated by furin, a subtilisin-like endoprotease. *EMBO J*. 1992;11:2407–2414.
14. Horimoto T, Kawaoka Y. Reverse genetics provides direct evidence for a correlation of hemagglutinin cleavability and virulence of an avian influenza A virus. *J Virol*. 1994;68:3120–3128.
15. Tong S, Li Y, Rivailler P, et al. A distinct lineage of influenza A virus from bats. *Proc Natl Acad Sci U S A*. 2012;109:4269–4274.
16. Ma W, Garcia-Sastre A, Schwemmle M. Expected and unexpected features of the newly discovered bat influenza A-like viruses. *PLoS Pathog*. 2015;11:e1004819.
17. Zhu X, Yang H, Guo Z, et al. Crystal structures of two subtype N10 neuraminidase-like proteins from bat influenza A viruses reveal a diverged putative active site. *Proc Natl Acad Sci U S A*. 2012;109:18903–18908.
18. Xu X, Lindstrom SE, Shaw MW, et al. Reassortment and evolution of current human influenza A and B viruses. *Virus Res*. 2004;103:55–60.
19. Chen W, Calvo PA, Malide D, et al. A novel influenza A virus mitochondrial protein that induces cell death. *Nat Med*. 2001;7:1306–1312.
20. Jagger BW, Wise HM, Kash JC, et al. An overlapping Protein-Coding Region in influenza A virus segment 3 modulates the host response. *Science*. 2012;337:199–204.
21. Shaw MI, Palese P. Orthomyxoviridae. In: Knipe DM, Howley P, eds. *Fields Virology*. Philadelphia, PA: Wolters Kluwer; 2013:1151–1185.
22. Simonsen L, Clarke MJ, Williamson DW, et al. The impact of influenza epidemics on mortality: introducing a severity index. *Am J Pub Health*. 1997;87:1944–1950.
23. Simonsen L, Taylor R, Viboud C, et al. US flu mortality estimates are based on solid science. *BMJ*. 2006;332:177–178.
24. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA*. 2003;289:179–186.
25. Reed C, Chaves SS, Daily Kirley P, et al. Estimating influenza disease burden from Population-Based Surveillance data in the United States. *PLoS ONE*. 2015;10:e0118369.
26. Chang DH, Bednarczyk RA, Becker ER, et al. Trends in U.S. hospitalizations and inpatient deaths from pneumonia and influenza, 1996-2011. *Vaccine*. 2016;34:486–494.
27. Sullivan KM, Monto AS, Longini IM. Estimates of the US health impact of influenza. *Am J Pub Health*. 1993;83:1712–1716.
28. Kavet J. A perspective on the significance of pandemic influenza. *Am J Pub Health*. 1977;67:1063–1070.
29. Nichol KL, Lind A, Margolis KL, et al. The effectiveness of vaccination against influenza in healthy, working adults. *N Engl J Med*. 1995;333:889–893.
30. Neuzil KM, Hohlbein C, Zhu Y. Illness among schoolchildren during influenza season: effect on school absenteeism, parental absenteeism from work, and secondary illness in families. *Arch Pediatr Adolesc Med*. 2002;156:986–991.
31. Keech M, Scott AJ, Ryan PJJ. The impact of influenza and influenza-like illness on productivity and healthcare resource utilization in a working population. *Occup Med*. 1998;48:85–90.
32. Barker WH, Borisute H, Cox C. A study of the impact of influenza on the functional status of frail older people. *Arch Intern Med*. 1998;158:645–650.
33. Glezen WP, Keitel WA, Taber LH, et al. Age distribution of patients with medically-attended illnesses caused by sequential variants of influenza A/H1N1: comparison to age-specific infection rates, 1978-1989. *Am J Epidemiol*. 1991;133:296–304.
34. Bhat N, Wright JG, Broder KR, et al. Influenza-Associated deaths among children in the United States, 2003-2004. *N Engl J Med*. 2005;353:2559–2567.
35. Ellis SE, Coffey CS, Mitchel EF Jr, et al. Influenza- and respiratory syncytial virus-associated morbidity and mortality in the nursing home population. *J Am Geriatr Soc*. 2003;51:761–767.
36. Siston AM, Rasmussen SA, Honein MA, et al. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. *JAMA*. 2010;303:1517–1525.
37. Neuzil KM, Reed GW, Mitchel EF, et al. The impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. *Am J Epidemiol*. 1998;148:1094–1102.
38. Louie JK, Acosta M, Jamieson DJ, et al. Severe 2009 H1N1 influenza in pregnant and postpartum women in California. *N Engl J Med*. 2010;362:27–35.
39. Mertz D, Geraci J, Winkup J, et al. Pregnancy as a risk factor for severe outcomes from influenza virus infection: a systematic review and meta-analysis of observational studies. *Vaccine*. 2017;35:521–528.
40. Forbes RL, Wark PAB, Murphy VE, et al. Pregnant women have attenuated innate interferon responses to 2009 pandemic influenza A virus subytpe H1N1. *J Infect Dis*. 2012;206:646–653.
41. Louie JK, Acosta M, Samuel MC, et al. A novel risk factor for a novel virus: obesity and 2009 pandemic influenza A (H1N1). *Clin Infect Dis*. 2011;52:301–312.
42. Kwong JC, Campitelli L, Rosella LC. Obesity and respiratory hospitalizations during influenza seasons in Ontario Canada: a cohort study. *Clin Infect Dis*. 2011;53:413–421.
43. Neuzil KM, Mellen BG, Wright PF, et al. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *N Engl J Med*. 2000;342:225–231.
44. Izurieta HS, Thompson WW, Kramarz P, et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. [see comments]. *N Engl J Med*. 2000;342:232–239.
45. Ampofo K, Gesteland PH, Bender J, et al. Epidemiology, complications, and cost of hospitalization in children with Laboratory-Confirmed influenza infection 10.1542/peds.2006-1475. *Pediatrics*. 2006;118:2409–2417.
46. Silberry GK. Complications of influenza infection in children. *Pediatr Ann*. 2000;29:683–690.
47. Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. *N Engl J Med*. 2006;355:31–40.
48. Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974-1976. *N Engl J Med*. 1978;298:587–593.
49. Monto AS, Kioumehr F. The Tecumseh study of respiratory illness. IX. Occurrence of influenza in the community, 1966-1971. *Am J Epidemiol*. 1975;102:553–559.
50. Azziz Baumgartner E, Dao CN, Nasreen S, et al. Seasonality, timing, and climate drivers of influenza activity worldwide. *J Infect Dis*. 2012;206:838–846.
51. Schaffer FL, Soergel ME, Straube DC. Survival of airborne influenza virus: effects of propagating host, relative humidity, and composition of spray fluids. *Arch Virol*. 1976;54:263–273.
52. Deyle ER, Maher MC, Hernandez RD, et al. Global environmental drivers of influenza. *Proc Natl Acad Sci USA*. 2016;113:13081–13086.
53. Lowen AC, Mubareka S, Steel J, et al. Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog*. 2007;3:e151.
54. Huang X, Mengersen K, Milinovich GJ, et al. Effect of weather variability on seasonal influenza among different age groups in Queensland, Australia: a bayesian spatiotemporal analysis. *J Infect Dis*. 2017;215:1695–1701.
55. Ewing A, Lee EC, Viboud C, et al. Contact, travel, and transmission: the impact of winter holidays on influenza dynamics in the United States. *J Infect Dis*. 2017;215:732–739.
56. Glezen WP, Couch RB, Six HR. The influenza herald wave. *Am J Epidemiol*. 1982;116:589–598.
57. Buxton-Bridges C, Kuehnert MJ, Hall CB. Transmission of influenza: implication for control in health care settings. *J Infect Dis*. 2003;37:1094–1101.
58. Blachere FM, Lindsley WG, Pearce TA, et al. Measurement of airborne influenza virus in a hospital emergency department. *Clin Infect Dis*. 2009;48:438–440.
59. Yang W, Elankumaran S, Marr L. Concentrations and size distributions of airborne influenza A viruses measured indoors at a health center, a day-care center, and on airplanes. *J R Soc Interface*. 2011;8:1176–1184.
60. Alford RH, Kasel JA, Gerone PJ, et al. Human influenza resulting from aerosol inhalation. *Proc Soc Exp Biol Med*. 1966;122:800–804.
61. Little JW, Douglas RG Jr, Hall WJ, et al. Attenuated influenza produced by experimental intranasal inoculation. *J Med Virol*. 1979;3:177–188.
62. Andrewes C, Glover R. Spread of infection from the respiratory tract of the ferret: I. Transmission of influenza A virus. *Br J Exp Pathol*. 1941;22:91–97.
63. Moser MR, Bender TR, Margolis HS, et al. An outbreak of influenza aboard a commercial airliner. *J Epidemiol*. 1979;110:1–6.
64. McLean R. The effect of ultraviolet radiation upon the transmission of epidemic influenza in long-term hospital patients. *Am Rev Respir Dis*. 1961;83.
65. Drinka PJ, Krause P, Schilling M, et al. Report of an outbreak: nursing home architecture and influenza A attack rates. *J Am Geriatr Soc*. 1996;44:910–913.
66. Kaiser L, Henry D, Flack NP, et al. Short-term treatment with zanamivir to prevent influenza: results of a placebo-controlled study. *Clin Infect Dis*. 2000;30:587–589.
67. Brankston G, Gitterman L, Hirji Z, et al. Transmission of influenza A in human beings. *Lancet Infect Dis*. 2007;7:257–265.
68. Loeb M, Dafoe N, Mahony J, et al. Surgical mask vs N95 respirators for preventing influenza among health care workers: a randomized trial. *JAMA*. 2009;302:1865–1871.
69. Cowling BJ, Chan K-H, Fang V, et al. Facemasks and hand hygiene to prevent influenza transmission in households. *Ann Intern Med*. 2009;151:437–446.
70. WHO. Pandemic Influenza Risk Management: WHO Interim Guidance. WHO/HSE/HEA/HSP/20133 2013.
71. Wilson IA, Cox NJ. Structural basis of immune recognition of influenza virus hemagglutinin. *Annu Rev Immunol*. 1990;8:737–771.
72. Webster RG, Laver WG. Determination of the number of nonoverlapping antigenic areas on Hong Kong (H3N2) influenza virus hemagglutinin with monoclonal antibodies and the selection of variants with potential epidemiological significance. *Virology*. 1980;104:139–148.
73. Xu X, Cox NJ, Bender CA, et al. Genetic variation in the neuraminidase genes of influenza A (H3N2) viruses. *Virology*. 1996;224:175–183.
74. Rimmelzwaan GF, Boon AC, Voeten JT, et al. Sequence variation in the influenza A virus nucleoprotein associated with escape from cytotoxic T lymphocytes. *Virus Res*. 2004;103:97–100.
75. Berkhoff EG, de Wit E, Geelhoed-Mieras MM, et al. Fitness costs limit escape from cytotoxic T lymphocytes by influenza A viruses. *Vaccine*. 2006;24:6594–6596.
76. Hay AJ, Gregory V, Douglas AR, et al. The evolution of human influenza viruses. *Philos Trans R Soc Lond B Biol Sci*. 2001;356:1861–1869.
77. Yamashita M, Krystal M, Fitch WM, et al. Influenza B virus evolution: co-circulating lineages and comparison of evolutionary patterns with those of influenza A and C viruses. *Virology*. 1988;163:112–122.
78. Smith DJ, Lapedes AS, de Jong JC, et al. Mapping the antigenic and genetic evolution of influenza virus. *Science*. 2004;305:371–376.
79. Kilbourne ED. *Influenza*. New York: Plenum Publishing; 1987.
80. Masurel N, Marine WM. Recycling of Asian and Hong Kong influenza A virus hemagglutinins in man. *Am J Epidemiol*. 1973;97:44–49.
81. Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. *J Virol*. 1989;63:4603–4608.
82. Claas ECJ, Osterhous ADME, van Beek R, et al. Human influenza A H5N1 related to a highly pathogenic avian influenza virus. *Lancet*. 1998;351:472–477.
83. WHO. Epidemiology of WHO-confirmed human cases of avian influenza A(H5N1) infection. *Wkly Epidemiol Rec*. 2005;81:249–257.

84. WHO. Avian influenza A (H5N1) infection in humans. *N Engl J Med*. 2005;353:1374–1385.
85. VanKerkhove MD, Mumford E, Mounts AW, et al. Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. *PLoS ONE*. 2011;6.
86. Olsen SJ, Ungchusak K, Sovann T, et al. Family clustering of avian influenza A (H5N1). *Emerg Infect Dis*. 2005;11:1799–1801.
87. Butler D. Pandemic "dry run" is cause for concern. *Nature*. 2006;441:554–555.
88. de Jong MD, Cam BV, Qui PT, et al. Fatal avian influenza A (H5N1) in a child presenting with diarrhea followed by coma. *N Engl J Med*. 2005;352:686–691.
89. Chotpitayasunondh T, Ungchusak K, Hanshaoworakul W, et al. Human disease from influenza A(H5N1), Thailand 2004. *Emerg Infect Dis*. 2005;11:201–209.
90. To K, Paul KS, Chan K-F, et al. Pathology of fatal human infection associated with avian influenza A H5N1 virus. *J Med Virol*. 2001;63:242–246.
91. Neumann G, Chen H, Gao GF, et al. H5N1 influenza viruses: outbreaks and biological properties. *Cell Res*. 2010;20:51–61.
92. Uyeki TM. Human infection with highly pathogenic avian influenza A (H5N1) virus: review of clinical issues. *Clin Infect Dis*. 2009;49:279–290.
93. Taylor HR, Turner AJ. A case report of fowl plague keratoconjunctivitis. *Br J Ophthalmol*. 1977;61:86–88.
94. Kurtz J, Manvell RJ, Banks J. Avian influenza virus isolated from a woman with conjunctivitis. *Lancet*. 1996;384:901–902.
95. Webster RG, Geraci J, Petursson G, et al. Conjunctivitis in human beings caused by influenza A virus of seals. *N Engl J Med*. 1981;304:911.
96. Koopmans M, Wilbrink B, Conyn M, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet*. 2004;363:587–593.
97. Fouchier RAM, Schneeberger PM, Rozendaal FW, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci U S A*. 2004;101:1356–1361.
98. Meijer A, Bosman A, van de Kamp EE, et al. Measurement of antibodies to avian influenza virus A (H7N7) in humans by hemagglutination inhibition test. *J Virol Meth*. 2006;132:113–120.
99. Hirst M, Astell CR, Griffith M, et al. Novel avian influenza H7N3 strain outbreak, British Columbia. *Emerg Infect Dis*. 2004;10:2192–2195.
100. Tweed SA, Skowronski DM, David ST, et al. Human illness from avian influenza H7N3, British Columbia. *Emerg Infect Dis*. 2004;10:2196–2199.
101. Nguyen Van Tam J, Nair P, Acheson P, et al. Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. *Euro Surveill*. 2006;11.
102. Zhu Y, Qi X, Cui L-B, et al. Human co-infection with novel avian influenza A H7N9 and influenza A H3N2 viruses in Jiangsu province, China. *Lancet*. 2013;381:2134.
103. Gao H-N, Lu H-Z, Cao B, et al. Clinical findings in 111 cases of influenza A (H7N9) virus infection. *N Engl J Med*. 2013;368:2277–2285.
104. Ai J, Huang Y, Xu K, et al. Case-control study of risk factors for human infection with influenza A(H7N9) virus in jiangsu province, China, 2013. *Euro Surveill*. 2013;18:20510.
105. Skowronski DM, Janjua NZ, Kwindt TL, et al. Virus-host interactions and the unusual age and sex distribution of human cases of influenza A(H7N9) in China, April 2013. *Euro Surveill*. 2013;18:20465.
106. Gostic KM, Ambrose M, Worobey M, et al. Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. *Science*. 2016;354:722–726.
107. Wang X, Jiang H, Wu P, et al. Epidemiology of avian influenza A H7N9 virus in human beings across five epidemics in mainland china, 2013-17: An epidemiological study of laboratory-confirmed case series. *Lancet Infect Dis*. 2017;17:822–832.
108. Burke DF, Trock SC. Use of Influenza Risk Assessment Tool for pandemic preparedness. *Emerg Infect Dis*. 2018;24:471–477.
109. Peiris M, Yuen KY, Leung CW, et al. Human infection with influenza H9N2. *Lancet*. 1999;354:916–917.
110. Guo YJ, Krauss S, Senne DA, et al. Characterization of the pathogenicity of members of the newly established H9N2 influenza virus lineages in Asia. *Virology*. 2000;267:279–288.
111. Beare AS, Webster RG. Replication of avian influenza viruses in humans. *Arch Virol*. 1991;119:37–42.
112. Naffakh N, Tomoiu A, Rameix-Welti MA, et al. Host restriction of avian influenza viruses at the level of the ribonucleoproteins. *Annu Rev Microbiol*. 2008;62:403–424.
113. Murphy BR, Buckler-White AJ, London WT, et al. Avian-human reassortant influenza A viruses derived by mating avian and human influenza A viruses. *J Infect Dis*. 1984;150:841–850.
114. Bean WJ, Schell M, Katz J, et al. Evolution of the H3 hemagglutinin from human and nonhuman hosts. *J Virol*. 1992;66:1129–1138.
115. Ito T, Couceiro JN, Kelm S, et al. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *J Virol*. 1998;72:7367–7373.
116. Scholtissek C, Stech J, Krauss S, et al. Cooperation between the hemagglutinin of avian viruses and the matrix protein of human influenza A viruses. *J Virol*. 2002;76:1781–1786.
117. Taubenberger JK, Reid AH, Krafft AE, et al. Initial genetic characterization of the 1918 "Spanish" influenza virus. *Science*. 1997;275:1793–1796.
118. Imai M, Watanabe T, Hatta M, et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature*. 2012;486:420–428.
119. Herfst S, Schrauwen EJA, Linster M, et al. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science*. 2012;336:1534–1541.
120. Russell CA, Fonville JM, Brown AEX, et al. The Potential for Respiratory Droplet–Transmissible A/H5N1 influenza virus to evolve in a mammalian host. *Science*. 2012;336:1541–1547.
121. Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of Swine-Origin 2009 a(H1N1) influenza viruses circulating in humans. *Science*. 2009;325:197–201.
122. Chou Y-Y, Albrecht RA, Pica N, et al. The M segment of the 2009 new pandemic H1N1 influenza virus is critical for its high transmission efficiency in the guinea pig model. *J Virol*. 2011;85:11235–11241.
123. Olsen CW. The emergence of novel swine influenza viruses in North America. *Virus Res*. 2002;85:199–210.
124. Vincent AL, Ma W, Lager KM, et al. Swine influenza viruses: a North American perspective. *Adv Virus Res*. 2008;72:127–154.
125. Lindstrom S, Garten R, Balish A, et al. Human infections with novel reassortant influenza A(H3N2)v viruses, United States, 2011. *Emerg Infect Dis*. 2012;18:834–837.
126. CDC. Update: influenza activity - United States and worldwide, May 20 - September 22,2012. *MMWR Morb Mortal Wkly Rep*. 2012;61:785–789.
127. Wong KK, Greenbaum A, Moll ME, et al. Outbreak of influenza A (H3N2) variant virus infection among attendees of an agricultural fair, Pennsylvania, USA, 2011. *Emerg Infect Dis*. 2012;18:1937–1944.
128. Skowronski DM, Janjua NZ, de Serres G, et al. Cross-reactive and vaccine-induced antibody to an emerging swine-origin variant of influenza A virus subtype H3N2 (H3N2v). *J Infect Dis*. 2012;206:1852–1861.
129. CDC. Antibodies cross-reactive to influenza A (H3N2) variant virus and impact of 2010-11 seasonal influenza vaccine on cross-reactive antibodies - United States. *MMWR Morb Mortal Wkly Rep*. 2012;61:237–241.
130. Howard JB. Influenza A2 virus as a cause of croup requiring tracheostomy. *J Pediatr*. 1972;81:1148–1150.
131. Glezen WP, Paredes A, Taber LH. Influenza in children. Relationship to other respiratory agents. *JAMA*. 1980;243:1345–1349.
132. Schwarzmann SW, Adler JL, Sullivan RFJ, et al. Bacterial pneumonia during the Hong Kong influenza epidemic of 1968-1969. *Arch Intern Med*. 1971;127:1037–1041.
133. Bisno AL, Griffin JP, VanEpps KA. Pneumonia and Hong Kong influenza: a prospective study of the 1968-1969 epidemic. *Am J Med Sci*. 1971;261:251–274.
134. CDC. Severe methicillin-resistant Staphylococcus aureus community-acquired pneumonia associated with influenza - Louisiana and Georgia, December 2006. *MMWR Morb Mortal Wkly Rep*. 2007;56:325–339.
135. Randolph AG, Ruifei X, Novak T, et al. Vancomycin monotherapy may be insufficient to treat methicillin-resistant *Staphylococcus aureus* coinfection in children with influenza-related critical illness. *Clin Infect Dis*. 2018;IN PRESS.
136. Ahn S, Kim WY, Kim SH, et al. Role of procalcitonin and C-reactive protein in differentiation of mixed bacterial infection from 2009 H1N1 viral pneumonia. *Influenza Other Respir Viruses*. 2011;5:398–403.
137. Piacentini E, Sanches B, Arauzo V, et al. Procalcitonin levels are lower in intensive care unit patients with H1N1 influenza A virus pneumonia than in those with community-acquired pneumonia. *J Crit Care*. 2011;26:201–205.
138. Kempe A, Hall CB, MacDonald NE, et al. Influenza in children with cancer. *J Pediatr*. 1989;115:33–39.
139. Whimbey E, Eling LS, Couch RB, et al. Influenza A virus infection among hospitalized adult bone marrow transplant recipients. *Bone Marrow Transplant*. 1994;13:437–440.
140. Yousuf HM, Englund J, Couch R, et al. Influenza among hospitalized adults with leukemia. *Clin Infect Dis*. 1997;24:1095–1099.
141. Klimov AI, Rocha E, Hayden FG, et al. Prolonged shedding of amantadine-resistant influenzae a viruses by immunodeficient patients: detection by polymerase chain reaction-restriction analysis. *J Infect Dis*. 1995;172:1352–1355.
142. Evans KM, Kline MW. Prolonged influenza A infection responsive to rimantadine therapy in a human immunodeficiency virus-infected child. *Pediatr Infect Dis J*. 1995;14:332–334.
143. Gubareva LV, Matrosovich MN, Brenner MK, et al. Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. *J Infect Dis*. 1998;178:1257–1262.
144. Ison MG, Gubareva LV, Atmar RL, et al. Recovery of Drug-Resistant influenza virus from immunocompromised patients: a case series. *J Infect Dis*. 2006;193:760–764.
145. Monto AS, Ross HW. The Tecumseh study of respiratory illness. X. relation of acute infections to smoking, lung function, and chronic symptoms. *Am J Epidemiol*. 1978;107:57–64.
146. Ferson MJ, Morton JR, Robertson PW. Impact of influenza on morbidity in children with cystic fibrosis. *J Paediatr Child Health*. 1991;27:308–311.
147. Crum-Cianflone NF. Bacterial, fungal, parasitic, and viral myositis. *Clin Microbiol Rev*. 2008;21:473–494.
148. Karjalainen J, Nieminen MS, Heikkila J. Influenza A1 myocarditis in conscripts. *Acta Med Scand*. 1980;207:27–30.
149. Craver RD, Sorrells K, Gohd R. Myocarditis with influenza B infection. *Pediatr Infect Dis J*. 1997;16:629–630.
150. Louria DB, Blumenfeld HL, Ellis JT, et al. Studies on influenza in the pandemic of 1957-1958. II. pulmonary complications of influenza. *J Clin Invest*. 1959;38:213–265.
151. Warren-Gash C, Hayward AC, Hemingway H, et al. Influenza infection and risk of acute myocardial infarction in England and Wales: a CALIBER self-controlled case series study. *J Infect Dis*. 2012;206:1652–1659.
152. Boilard E, Pare G, Rousseau M, et al. Influenza virus H1N1 activates platelets through FcgammaRIIA signaling and thrombin generation. *Blood*. 2014;123:2854–2863.
153. Kwong JC, Schwartz KL, Campitelli MA, et al. Acute myocardial infarction after laboratory-confirmed influenza infection. *N Engl J Med*. 2018;378:345–353.
154. MacDonald KL, Osterholm MT, Hedberg CW, et al. Toxic shock syndrome: a newly recognized complication of influenza and influenza like illness. *J Am Med Assoc*. 1987;257:1053–1058.
155. Sperber SJ, Francis JB. Toxic shock during an influenza outbreak. *J Am Med Assoc*. 1987;257:1086–1089.
156. Kashiwada T, Kikuchi K, Abe S, et al. Staphylococcal enterotoxin B toxic shock syndrome induced by community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA). *Intern Med*. 2012;51:3085–3088.
157. Edelen JS, Bender TR, Chin TDY. Encephalopathy and pericarditis during an outbreak of influenza. *Am J Epidemiol*. 1974;100:79–83.
158. Steininger C, Popow-Kraupp T, Laferl H, et al. Acute encephalopathy associated with influenza A virus infection. *Clin Infect Dis*. 2003;36:567–574.
159. Britton PN, Blyth CC, Macartney K, et al. The spectrum and burden of influenza-associated neurological disease in children: combined encephalitis and influenza sentinel site surveillance from Australia, 2013-2015. *Clin Infect Dis*. 2017;65:653–660.
160. Okuno H, Yahata Y, Tanaka-Taya K, et al. Characteristics and outcomes of influenza-associated encephalopathy cases among children and adults in Japan, 2010-2015. *Clin Infect Dis*. 2018;66:1831–1837.
161. Sanz-Esquerro JJ, De La Luna S, Ortin J, et al. Individual expression of the influenza virus PA protein induces degradation of coexpressed proteins. *J Virol*. 1995;69:2420–2426.
162. Hinshaw VS, Olsen CW, Dybdahl-Sissoko N, et al. Apoptosis: a mechanism of cell killing by influenza A and B viruses. *J Virol*. 1994;68:3667–3673.
163. Mori I, Komatsu T, Takeuchi K, et al. In vivo induction of apoptosis by influenza virus. *J Gen Virol*. 1995;76:2869–2873.
164. Takizawa T, Fukuda R, Miyawaki T, et al. Activation of the apoptotic Fas antigen-encoding gene upon influenza virus infection involving spontaneously produced beta-interferon. *Virology*. 1995;209:288–296.

165. Jordan WS, Badger GF, Dingle JH. A study of illness in a group of Cleveland families. XVI. The epidemiology of influenza 1948-1953. *Am J Hyg*. 1958;68:169–189.
166. Larson HE, Parry RP, Tyrrell DAJ. Impaired polymorphonuclear leucocyte chemotaxis after influenza virus infection. *Br J Dis Chest*. 1980;74:56–62.
167. Roberts NJ, Steigbigel RT. Effect of in vitro virus infection on response of human monocytes and lymphocytes to mitogen stimulation. *J Immunol*. 1978;121:1052–1058.
168. Cooper JA Jr, Carcelen R, Culbreth R. Effects of influenza A nucleoprotein on polymorphonuclear neutrophil function. *J Infect Dis*. 1996;173:279–284.
169. Cooper JA Jr, Culbreth RR. Characterization of a neutrophil inhibitor peptide harvested from human bronchiolar lavage: homology to influenza A nucleoprotein. *Am J Respir Cell Mol Biol*. 1996;15:207–215.
170. Abramson JS, Wheeler JG, Parce JW, et al. Suppression of endocytosis in neutrophils by influenza A virus in vitro. *J Infect Dis*. 1986;154:456–463.
171. Hayden FG, Fritz R, Lobo MC, et al. Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. *J Clin Invest*. 1998;101:643–649.
172. Lau LLH, Dennis KMI, Nishiura H, et al. Heterogeneity in viral shedding among individuals with medically attended influenza A virus infection. *J Infect Dis*. 2013;207:1281–1285.
173. Choi S-M, Xie H, Campbell AP, et al. Influenza viral RNA detection in blood as a marker to predict disease severity in hematopoietic cell transplant recipients. *J Infect Dis*. 2017;206:1872–1877.
174. Peiris JSM, Yu WC, Leung CW, et al. Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet*. 2004;363:617–619.
175. de Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med*. 2006;12:1203–1207.
176. Everitt AR, Clare S, Pertel T, et al. IFITM3 restricts the morbidity and mortality associated with influenza. *Nature*. 2012;484:519.
177. Wang Z, Zhang A, Wan Y, et al. Early hypercytokinemia is associated with interferon-induced transmembrane protein-3 dysfunction and predictive of fatal H7N9 infection. *Proc Natl Acad Sci U S A*. 2014;111:769–774.
178. Allen EK, Randolph AG, Bhangale T, et al. SNP-mediated disruption of CTCF binding at the IFITM3 promoter is associated with risk of severe influenza in humans. *Nat Med*. 2017;23:975.
179. Ciancanelli MJ, Huang SXL, Luthra P, et al. Life-threatening influenza and impaired interferon amplification in human IRF7 deficiency. *Science*. 2015;348:448–453.
180. Lee N, Cao B, Ke C, et al. IFITM3, TLR3, and CD55 gene SNPs and cumulative genetic risks for severe outcomes in Chinese patients with H7N9/h1n1pdm09 influenza. *J Infect Dis*. 2017;216:97–104.
181. Walsh KB, Teijaro JR, Wilker PR, et al. Suppression of cytokine storm with a sphingosine analog provides protection against pathogenic influenza virus. *Proc Natl Acad Sci U S A*. 2011;108:12018–12023.
182. Rodrigo C, Leonardi-Bee J, Nguyen-Van Tam JS, et al. Effect of corticosteroid therapy on influenza-related mortality: a systemic review and meta-analysis. *J Infect Dis*. 2015;212:183–194.
183. Vandermeer ML, Thomas AR, Kamimoto L, et al. Association between use of statins and mortality among patients hospitalized with laboratory-confirmed influenza virus infections: a multistate study. *J Infect Dis*. 2012;205:13–19.
184. Fedson DS. Treating influenza with statins and other immunomodulatory agents. *Antiviral Res*. 2013;99:417–435.
185. Martin CM, Kunin CM, Gottlieb LS, et al. Asian influenza A in Boston, 1957-1958. *Arch Intern Med*. 1959;103:516–531.
186. Walsh JJ, Dietlein LF, Low FN, et al. Bronchotracheal response in human influenza. *Arch Intern Med*. 1961;108:376–388.
187. Guarner J, Shieh WJ, Dawson J, et al. Immunohistochemical and in situ hybridization studies of influenza A virus infection in human lungs. *Am J Clin Pathol*. 2000;114:227–233.
188. Oseasohn R, Adelson L, Kaji M. Clinicopathologic study of thirty-three fatal cases of Asian influenza. *N Engl J Med*. 1959;11:509–518.
189. Little JW, Hall WJ, Douglas RG Jr, et al. Airway hyperreactivity and peripheral airway dysfunction in influenza A infection. *Am Rev Respir Dis*. 1978;118:295–303.
190. Hall WJ, Douglas RG Jr, Hyde RW, et al. Pulmonary mechanics after uncomplicated influenza A infection. *Am Rev Respir Dis*. 1976;113:141–147.
191. Horner GJ, Gray FD Jr. Effect of uncomplicated, presumptive influenza on the diffusing capacity of the lung. *Am Rev Respir Dis*. 1973;108:866–869.
192. Johanson WGJ, Pierce AK, Sanford JP. Pulmonary function in uncomplicated influenza. *Am Rev Respir Dis*. 1969;100:141–146.
193. Kondo S, Abe K. The effects of influenza virus infection on FEV1 in asthmatic children. *Chest*. 1991;100:1235–1238.
194. Smith CB, Kanner RE, Goldern CA, et al. Effect of viral infections on pulmonary function in patients with chronic obstructive pulmonary diseases. *J Infect Dis*. 1980;141:271–279.
195. Utell MJ, Aquilina AT, Hall WJ, et al. Development of airway reactivity to nitrates in subjects with influenza. *Am Rev Respir Dis*. 1980;121:233–241.
196. Yelandi AV, Colby TV. Pathologic features of lung biopsy specimens from influenza pneumonia cases. *Hum Pathol*. 1994;25:47–53.
197. Kash JC, Taubenberger JK. The role of viral, host, and secondary bacterial factors in influenza pathogenesis. *Am J Pathol*. 2015;185:1528–1536.
198. Levandowski RA, Gerrity TR, Garrard CS. Modifications of lung clearance mechanisms by acute influenza A infection. *J Lab Clin Med*. 1985;106:428–432.
199. Pittet LA, Hall-Stoodley L, Rutkowski MR, et al. Influenza virus infection decreases tracheal mucociliary velocity and clearance of streptococcus pneumonia. *Am J Respir Cell Mol Biol*. 2010;42:450–460.
200. Li W, Moltedo B, Moran TM. Type I interferon induction during influenza virus infection increases susceptibility to secondary *Streptococcus pneumoniae* infection by negative regulation of T cells. *J Virol*. 2012;86:12304–12312.
201. Tian X, Xu F, Lung WY, et al. Poly I:C enhances susceptibility to secondary pulmonary infections by gram-positive bacteria. *PLoS ONE [Electronic Resource]*. 2012;7:e41879.
202. van der Sluijs KF, van Elden LJ, Nijhuis M, et al. IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection. *J Immunol*. 2004;172:7603–7609.
203. Sun K, Metzger DW. Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection. *Nat Med*. 2008;14:558–564.
204. Sun K, Metzger DW. Influenza infection suppresses NADPH oxidase-dependent phagocytic bacterial clearance and enhances susceptibility to secondary methicillin-resistant *Staphylococcus aureus* infection. *J Immunol*. 2014;192:3301–3307.
205. Small CL, Shaler CR, McCormick S, et al. Influenza infection leads to increased susceptibility to subsequent bacterial superinfection by impairing NK cell responses in the lung. *J Immunol*. 2010;184:2048–2056.
206. Raftogiannis M, Antonopoulou A, Baziaka F, et al. Indication for a role of regulatory T cells for the advent of influenza A (H1N1)-related pneumonia. *Clin Exp Immunol*. 2010;161:576–583.
207. Epstein SL. Prior H1N1 influenza infection and susceptibility of Cleveland family study participants during the H2N2 pandemic of 1957: and experiment of nature. *J Infect Dis*. 2006;193:49–53.
208. Black RA, Rota PA, Gorodkova N, et al. Antibody response to the M2 protein of influenza A virus expressed in insect cells. *J Gen Virol*. 1993;74:143–146.
209. Murphy BR, Kasel JA, Chanock RM. Association of serum antineuraminidase antibody with resistance to influenza in man. *N Engl J Med*. 1972;286:1329–1332.
210. Babu TM, Perera RAPM, Wu JT, et al. Population serologic immunity to human and avian H2N2 viruses in the United States and Hong Kong for pandemic risk assessment. *J Infect Dis*. 2018;IN PRESS.
211. Virelizier J-L. Host defenses against influenza virus: the role of anti-hemagglutinin antibody. *J Immunol*. 1975;115:434–439.
212. Black S, Nicolay U, Mesikari T, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. *Pediatr Infect Dis J*. 2011;30:1081–1085.
213. Stephenson I, Das RG, Wood JM, et al. Comparison of neutralising antibody assays for detection of antibody to influenza A/H3N2 viruses: an international collaborative study. *Vaccine*. 2007;25:4056–4063.
214. Kashyap AK, Steel J, Oner AF, et al. Combinatorial antibody libraries from survivors of the Turkish H5N1 avian influenza outbreak reveal virus neutralization strategies. *Proc Natl Acad Sci U S A*. 2008;105:5986–5991.
215. Wrammert J, Koutsnanos D, Li G-M, et al. Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med*. 2011;208:191.
216. Ekiert DC, Bhabha G, Elsliger M-A, et al. Antibody recognition of a highly conserved influenza virus epitope. *Science*. 2009;324:246–251.
217. Ekiert DC, Friesen RHE, Bhabha G, et al. A highly conserved neutralizing epitope on group 2 influenza A viruses. *Science*. 2011;333:843–850.
218. Pica N, Palese P. Towards a universal influenza virus vaccine: prospects and challenges. *Annu Rev Med*. 2013;64:189–202.
219. Webster RG, Reay PA, Laver WG. Protection against lethal influenza with neuraminidase. *Virology*. 1988;164:230–237.
220. Schulman JL, Khakpour M, Kilbourne ED. Protective effects of specific immunity to viral neuraminidase on influenza virus infection of mice. *J Virol*. 1968;2:778–786.
221. Schulman JL, Khakpour M, Kilbourne ED. Protective effects of hemagglutinin and neuraminidase antigens on influenza virus: distinctiveness of hemagglutinin antigens of Hong Kong - 68 virus. *J Virol*. 1968;2:778.
222. Monto AS, Kendal AP. Effect of neuraminidase antibody on Hong Kong influenza. *Lancet*. 1973;7804:623–625.
223. Clements ML, Betts RF, Tierney EL, et al. Resistance of adults to challenge with influenza A wild-type virus after receiving live or inactivated virus vaccine. *J Clin Microbiol*. 1986;23:73–76.
224. Memoli MJ, Shaw PA, Han A, et al. Evaluation of antihemagglutinin and antineuraminidase antibodies as correlates of protection in an influenza A/H1N1 virus healthy human challenge model. *MBio*. 2015;7:e00417–16.
225. Johansson BE, Grajower B, Kilbourne ED. Infection-permissive immunization with influenza virus neuraminidase prevents weight loss in infected mice. *Vaccine*. 1993;11:1037–1039.
226. Treanor JJ, Tierney EL, Zebedee SL, et al. Passively transferred monoclonal antibody to the M2 protein inhibits influenza A virus replication in mice. *J Virol*. 1990;64:1375–1377.
227. Jegerlehner A, Schmitz N, Storni T, et al. influenza A vaccine based on the extracellular domain of m2: weak protection mediated via antibody-dependent NK cell activity. *J Immunol*. 2004;172:5598–5605.
228. Carragher DM, Kaminski DA, Moquin A, et al. A novel role for non-neutralizing antibodies against nucleoprotein in facilitating resistance to influenza virus. *J Immunol*. 2008;181:4168–4176.
229. Wagner DK, Clements ML, Reimer CB, et al. Analysis of immunoglobulin G antibody responses after administration of live and inactivated influenza A vaccine indicates that nasal wash immunoglobulin G is a transudate from serum. *J Clin Microbiol*. 1987;25:559–562.
230. Murphy BR, Clements ML. The systemic and mucosal immune response of humans to influenza A virus. *Curr Top Microbiol Immunol*. 1989;146:107–116.
231. Renegar KB, Small PAJ. Passive transfer of local immunity to influenza virus by IgA antibody. *J Immunol*. 1991;146:1972–1978.
232. Liew FY, Russell SM, Appleyard G, et al. Cross-protection in mice infected with influenza A virus by the respiratory route is correlated with local IgA antibody rather than serum antibody or cytotoxic T cell activity. *Eur J Immunol*. 1984;14:409–413.
233. Clements ML, Betts RF, Tierney EL, et al. Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. *J Clin Microbiol*. 1986;24:157–160.
234. Belshe RB, Gruber WC, Mendelman PM, et al. Correlates of immune protection induced by live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. *J Infect Dis*. 2000;181:1133–1137.
235. Treanor J, Wright PF. Immune correlates of protection against influenza in the human challenge model. *Dev Biol (Basel)*. 2003;115:97–105.
236. Hillaire ML, van Trierum SE, Bodewes R, et al. Characterization of the human CD8+ T-cell response following infection with 2009 pandemic influenza H1N1 virus. *J Virol*. 2011;85:12057–12061.
237. Sasaki S, Jaimes MC, Holmes TH, et al. Comparison of the influenza-specific effector and memory B cell responses to immunization of children and adults with live attenuated or inactivated influenza vaccines. *J Virol*. 2007;81:215–228.
238. Kosor Krnic E, Gagro A, Drazenovic V, et al. Enumeration of haemagglutinin-specific CD8+ T cells after influenza vaccination using MHC class I peptide tetramers. *Scand J Immunol*. 2008;67:86–94.
239. Gooskens J, Jonges M, Claas EC, et al. Prolonged influenza virus infection during lymphocytopenia and frequent detection of drug-resistant viruses. *J Infect Dis*. 2009;199.

240. McElhaney JE, Ewen C, Zhou X, et al. Granzyme B: correlates with protection and enhanced CTL response to influenza vaccination in older adults. *Vaccine*. 2009;27:2418–2425.
241. McMichael AJ, Gotch FM, Noble GR, et al. Cytotoxic T-cell immunity to influenza. *N Engl J Med*. 1983;309:13–17.
242. Wilkinson TM, Li CKF, Chui CSC, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. *Nat Med*. 2012;18:274–280.
243. Forrest BD, Pride MW, Dunning AJ, et al. Correlation of cellular immune responses with protection against culture-confirmed influenza virus in young children. *Clin Vaccine Immunol*. 2008;15:1042–1053.
244. Sridhar S, Begom S, Bermingham A, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. *Nat Med*. 2013;19:1305–1312.
245. Hayward AC, Wang L, Goonetilleke N, et al. Natural T cell-mediated protection against seasonal and pandemic influenza: results of the flu watch cohort study. *Am J Resp Crit Care Med*. 2015;191:1422–1431.
246. Wang Z, Wan Y, Qiu C, et al. Recovery from severe H7N9 disease is associated with diverse response mechanisms dominated by CD8+ T cells. *Nat Commun*. 2015;6:6833.
247. Boivin G, Hardy I, Tellier G, et al. Predicting influenza infections during epidemics with use of a clinical case definition. *Clin Infect Dis*. 2000;31:1166–1169.
248. Monto AS, Gravenstein S, Elliott M, et al. Clinical signs and symptoms predicting influenza infection. *Arch Intern Med*. 2000;160:3243–3247.
249. Zambon M, Hays J, Webster A, et al. Diagnosis of influenza in the community: relationship of clinical diagnosis to confirmed virological, serologic, or molecular detection of influenza. *Arch Intern Med*. 2001;161:2116–2122.
250. Walsh EE, Cox C, Falsey AR. Clinical features of influenza A virus infection in older hospitalized persons. *J Am Geriatr Soc*. 2002;50:1498–1503.
251. Ruest A, Michaud S, Deslandes S, et al. Comparison of the directigen flu a+B test, the QuickVue influenza test, and clinical case definition to viral culture and reverse transcription-PCR for rapid diagnosis of influenza virus infection. *J Clin Microbiol*. 2003;41:3487–3493.
252. Falsey AR. Noninfluenza respiratory virus infection in long-term care facilities. *Infect Control Hosp Epidemiol*. 1991;12:602–608.
253. Drinka PJ, Gravenstein S, Krause P, et al. Non-influenza respiratory viruses may overlap and obscure influenza activity. *J Am Geriatr Soc*. 1999;47:1087–1093.
254. Coiras MT, Perez-Brena P, Garcia ML, et al. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. *J Med Virol*. 2003;69:132–144.
255. Notomi T, Okayama H, Masubuchi H, et al. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res*. 2000;28:e63.
256. Bell J, Boner A, Cohen DM, et al. Multicenter clinical evaluation of the novel Alere i influenza A&B isothermal nucleic acid amplification test. *J Clin Virol*. 2014;61:81–86.
257. Covalciuc KA, Webb KH, Carlson CA. Comparison of four clinical specimen types for detection of influenza A and B viruses by optical immunoassay (FLU OIA test) and cell culture methods. *J Clin Microbiol*. 1999;37:3971–3974.
258. Ryan-Pourier KA, Katz JM, Webster RG, et al. Application of directagen FLU-A for the detection of influenza A virus in human and non-human specimens. *J Clin Microbiol*. 1992;30:1072–1075.
259. Merckx J, Wali R, Schiller I, et al. Diagnostic accuracy of novel and traditional rapid tests for influenza infection compared with reverse transcriptase polymerase chain reaction: a systematic review and meta-analysis. *Ann Intern Med*. 2017;167:394–409.
260. Newton DW, Mellen CF, Baxter BD, et al. Practical and sensitive screening strategy for detection of influenza virus. *J Clin Microbiol*. 2002;40:4353–4356.
261. Harper SA, Bradley JS, Englund JA, et al. Seasonal influenza in adults and children—diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the infectious diseases society of America. *Clin Infect Dis*. 2009;48:1003–1032.
262. Belongia EA, Simpson MD, King JP, et al. Variable influenza vaccine effectiveness by subtype: a systematic review and meta-analysis of test-negative design studies. *Lancet Infect Dis*. 2016;16:942–951.
263. Kilbourne ED, Schulman JL, Schild GC, et al. Correlated studies of a recombinant influenza-virus vaccine. I. derivation and characterization of virus and vaccine. *J Infect Dis*. 1971;124:449–462.
264. Robertson JS, Bootman JS, Newman R, et al. Structural changes in the haemagglutinin which accompany egg adaptation of an influenza A (H1N1) virus. *Virology*. 1987;160:31–37.
265. Skowronski DM, Janjua NZ, De Serres G, et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. *PLoS ONE [Electronic Resource]*. 2014;9:e92153.
266. Linderman SL, Chambers BS, Zost SJ, et al. Potential antigenic explanation for atypical H1N1 infections among middle-aged adults during the 2013–2014 influenza season. *Proc Natl Acad Sci USA*. 2014;111:15798–15803.
267. Zost SJ, Parkhouse K, Gumina ME, et al. Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains. *Proc Natl Acad Sci USA*. 2017;114:12578–12583.
268. Maassab HF. Biologic and immunologic characteristics of cold-adapted influenza virus. *J Immunol*. 1969;102:728–732.
269. France EK, Glanz JM, Xu S, et al. Safety of the trivalent inactivated influenza vaccine among children - A population-based study. *Arch Pediatr Adolesc Med*. 2004;158:1031–1036.
270. Govaert TM, Thijs CT, Masurel N, et al. The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial. *J Am Med Assoc*. 1994;272:1956–1961.
271. Nichol KL, Margolis KL, Lind A, et al. Side effects associated with influenza vaccination in healthy working adults. A randomized, placebo-controlled trial. *Arch Intern Med*. 1996;156:1546–1550.
272. Chung EY, Huang L, Schneider L. Safety of influenza vaccine administration in egg-allergic patients. *Pediatrics*. 2010;125:e1024–e1030.
273. Grohskopf LA, Sokolow LZ, Broder KR, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the advisory committee on immunization practices - United States, 2017-18 influenza season. *MMWR Recomm Rep*. 2017;66:1–20.
274. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, et al. Guillain-Barré syndrome following vaccination in the national influenza immunization program, United States, 1976-1977. *Am J Epidemiol*. 1979;110:105–123.
275. Lasky T, Tarracciano GJ, Magder L, et al. The Guillain-Barré syndrome and the 1992-1993 and 1993-1994 influenza vaccines. *N Engl J Med*. 1998;339:1797–1802.
276. Martin Arias LH, Sanz R, Sainz M, et al. Guillain-Barré syndrome and influenza vaccines: a meta-analysis. *Vaccine*. 2015;33:3773–3778.
277. Scheifele DW, Dubal B, Russell ML, et al. Ocular and respiratory symptoms attributable to inactivated split influenza vaccine: evidence from a controlled trial involving adults. *Clin Infect Dis*. 2003;36:850–857.
278. Tamma PD, Sult KS, delRio D, et al. Safety of influenza vaccination during pregnancy. *Am J Obstet Gynecol*. 2009;547–553.
279. Nohynek H, Jokinen J, Partinen M, et al. AS03 adjuvanted AH1n1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. *PLoS ONE*. 2012;7:e33536.
280. Miller E, Andrews NJ, Stellitano L, et al. Risk of narcolepsy in children and young people receiving AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine: retrospective analysis. *BMJ*. 2013;346:f794.
281. Szakacs A, Darin N, Hallbook T. Increased childhood incidence of narcolepsy in western Sweden after H1N1 vaccination. *Neurology*. 2013;80:1315–1321.
282. Dauvilliers Y, Arnulf I, Mignot E. Narcolepsy with cataplexy. *Lancet*. 2007;369:499–511.
283. Partinen M, Saarenpaa-Heikkila O, Ilveskoski I, et al. Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. *PLoS ONE*. 2012;7:e33723.
284. Han F, Lin L, Warby SC, et al. Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in china. *Ann Neurol*. 2011;70:410–417.
285. Wijnans L, Lecomte C, de Vries C, et al. The incidence of narcolepsy in Europe: before, during, and after the influenza A(H1N1)pdm09 pandemic and vaccination campaigns. *Vaccine*. 2013;31:1246–1254.
286. Tsai TF, Crucitti A, Nacci P, et al. Explorations of clinical trials and pharmacovigilance databases of MF59®-adjuvanted influenza vaccines for associated cases of narcolepsy. *Scand J Infect Dis*. 2011;43:702–706.
287. Murphy BR, Coelingh K. Principles underlying the development and use of live attenuated cold-adapted influenza A and B virus vaccines. *Viral Immunol*. 2002;15:295–323.
288. Jin H, Lu B, Zhou H, et al. Multiple amino acid residues confer temperature sensitivity to human influenza virus vaccine strains (FluMist) derived from cold-adapted A/Ann Arbor/6/60. *Virology*. 2003;306:18–24.
289. Snyder MH, Betts RF, DeBorde D, et al. Four viral genes independently contribute to attenuation of live influenza A/Ann Arbor/6/60 (H2N2) cold-adapted reassortant virus vaccines. *J Virol*. 1988;62:488–495.
290. Subbarao EK, Perkins M, Treanor JJ, et al. The attenuation phenotype conferred by the M gene of the influenza A/Ann Arbor/6/60 cold-adapted virus (H2N2) on the A/Korea/82 (H3N2) reassortant virus results from a gene constellation effect. *Virus Res*. 1992;25:37–50.
291. Chen Z, Aspelund A, Kemble G, et al. Genetic mapping of the cold-adapted phenotype of B/ann Arbor/1/66, the master donor virus for live attenuated influenza vaccines (FluMist(R). *Virology*. 2006;345:416–423.
292. Hoffman E, Mahmood K, Chen Z, et al. Multiple gene segments control the temperature sensitivity and attenuation phenotypes of ca B/ann Arbor/1/66. *J Virol*. 2005;79:11014–11021.
293. Buonagurio DA, O'Neill RE, Shutyak L, et al. Genetic and phenotypic stability of cold-adapted influenza viruses in a trivalent vaccine administered to children in a day care setting. *Virology*. 2006;347:296–306.
294. Belshe RB, Edwards KM, Vesikari T, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. *N Engl J Med*. 2007;356:685–696.
295. Redding G, Walker RE, Helssel C, et al. Safety and tolerability of cold-adapted influenza virus vaccine in children and adolescents with asthma. *Pediatr Infect Dis J*. 2002;21:44–48.
296. Gorse GJ, Belshe RB, Munn NJ. Local and systemic antibody responses in high-risk adults given live attenuated and inactivated influenza A virus vaccines. *J Clin Microbiol*. 1988;26:911–918.
297. Atmar RL, Bloom K, Keitel W, et al. Effect of live attenuated, cold recombinant (CR) influenza virus vaccines on pulmonary function in heathy and asthmatic adults. *Vaccine*. 1990;8:217–224.
298. Jackson LA, Holmes SJ, Mendelman PM, et al. Safety of a trivalent live attenuated intranasal vaccine, FluMist, administered in addition to parenteral trivalent inactivated influenza vaccine to seniors with chronic medical conditions. *Vaccine*. 1999;17:1905–1909.
299. King JC, Treanor J, Fast PE, et al. Comparison of the safety, vaccine virus shedding, and immunogenicity of influenza virus vaccine, trivalent, types A and B, live cold-adapted, administered to human immunodeficiency virus (HIV)-infected and non-HIV-infected adults. *J Infect Dis*. 2000;181:725–728.
300. King JC Jr, Fast PE, Zangwill KM, et al. Safety, vaccine virus shedding and immunogenicity of trivalent, cold-adapted, live attenuated influenza vaccine administered to human immunodeficiency virus-infected and noninfected children. *Pediatr Infect Dis J*. 2001;20:1124–1131.
301. Talbot TR, Crocker DD, Peters J, et al. Duration of virus shedding after trivalent intranasal live attenuated influenza vaccination in adults. *Infect Control Hosp Epidemiol*. 2005;26:494–500.
302. Vesikari T, Karvonen A, Korhonen T, et al. A randomized, double-blind study of the safety, transmissibility, and phenotypic and genotypic stability of cold-adapted influenza virus vaccine. *Pediatr Infect Dis J*. 2006;25:590–597.
303. Wright PF, Thompson J, Vaughn WT, et al. Trials of influenza A/New Jersey/76 virus vaccine in normal children: an overview of age-related antigenicity and reactogenicity. *J Infect Dis*. 1977;136:S731–S741.
304. Wright PF, Cherry JD, Foy HM, et al. Antigenicity and reactogenicity of influenza A/USSR/77 virus vaccine in children - a multicentered evaluation of dosage and toxicity. *Rev Infect Dis*. 1983;5:758–764.
305. Levandowski RA, Regnery HL, Staton E, et al. Antibody responses to influenza B viruses in immunologically unprimed children. *Pediatrics*. 1991;88:1031–1036.
306. Lerman SJ, Wright PJ, Patil KD. Antibody decline in children following A/New Jersey/76 influenza virus immunization. *J Pediatr*. 1980;96:271–274.
307. Zahradnik JM, Kasel JA, Martin RR, et al. Immune responses in serum and respiratory secretions following vaccination with a live cold-recombinant (CR35) and inactivated A/USSR/77 (H1N1) influenza virus vaccine. *J Med Virol*. 1983;11:277–285.
308. Bokstad KA, Eriksson J-C, Cox RJ, et al. Parenteral vaccination against influenza does not induce a local antigen-specific immune response in the nasal mucosa. *J Infect Dis*. 2002;185:878–884.
309. Keitel WA, Couch RB, Cate TR, et al. High doses of purified influenza A virus hemagglutinin significantly augment serum and nasal secretion antibody responses in

healthy young adults. *J Clin Microbiol*. 1994;32:2468–2473.

310. Brokstad KA, Eriksson J-C, Cox RJ, et al. Parenteral vaccination against influenza does not induce a local antigen-specific immune response in the nasal mucosa. *J Infect Dis*. 2002;185:878–885.
311. Cox RJ, Brokstad KA, Zuckerman MA, et al. An early humoral immune response in peripheral blood following parenteral influenza vaccination. *Vaccine*. 1994;12:993–999.
312. el-Madhun AS, Cox RJ, Soreide A, et al. Systemic and mucosal immune response in young children and adults after parenteral influenza vaccination. *J Infect Dis*. 1998;178:933–939.
313. Danke NA, Kwok WW. HLA class II=restricted CD4+ T cell responses directed against influenza viral antigens postinfluenza vaccination. *J Immunol*. 2003;171:3163–3169.
314. Nayak JL, Fitzgerald T, Richards KA, et al. CD4 T-cell expansion predicts neutralizing antibody responses to monovalent inactivated pandemic H1N1 influenza vaccine. *J Infect Dis*. 2013;207:297–305.
315. Bentebibel S-E, Lopez S, Obermoser G, et al. Induction of CCOS CXR3 CXR5 TH cells correlates with antibody responses to influenza vaccination. *Sci Transl Med*. 2013;5:76ra32.
316. Zeman AM, Holmes TH, Stamatis S, et al. Humoral and cellular immune responses in children given annual immunization with trivalent inactivated influenza vaccine. *Pediatr Infect Dis J*. 2007;26:107–115.
317. McElhaney JE, Xie D, Hager WD, et al. T cell responses are better correlates of vaccine protection in the elderly. *J Immunol*. 2006;176:6333–6339.
318. Englund JA, Walter EB, Fairchok MP, et al. A comparison of 2 influenza vaccine schedules in 6- to 23-Month-Old Children. *Pediatrics*. 2005;115:1039–1047.
319. Englund JA, Walter EB, Gbadebo A, et al. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. *Pediatrics*. 2006;118:e579–e585.
320. Gross PA, Weksler ME, Quinnan GVJ, et al. Immunization of elderly people with two doses of influenza vaccine. *J Clin Microbiol*. 1987;25:1763–1765.
321. Hopping AM, McElhaney JE, Fonville JM, et al. The confounded effects of age and exposure history in response to influenza vaccination. *Vaccine*. 2016;34:540–546.
322. Falsey AR, Treanor JJ, Tornieporth N, et al. Superior immunogenicity of high dose influenza vaccine compared with standard influenza vaccine in adults > 65 years of age: a randomized double-blinded controlled phase 3 trial. *J Infect Dis*. 2008;200:172–180.
323. Cate TR, Rayford Y, Nino D, et al. A high dosage influenza vaccine induced significantly more neuraminidase antibody than standard vaccine among elderly subjects. *Vaccine*. 2010;28:2076–2078.
324. Frey SE, Poland GA, Percell S, et al. Comparison of the safety, tolerability, and immunogenicity of a MF59-adjuvanted influenza vaccine and a non-adjuvanted vaccine in non-elderly adults. *Vaccine*. 2003;21:4234–4237.
325. Podda A. The adjuvanted influenza vaccines with novel adjuvants: experience with the MF59-adjuvanted vaccine. *Vaccine*. 2001;19:2673–2680.
326. Frey SE, Reyes MRA-DL, Reynales H, et al. Comparison of the safety and immunogenicity of an MF59®-adjuvanted with a non-adjuvanted seasonal influenza vaccine in elderly subjects. *Vaccine*. 2014;32:5027–5034.
327. Duchini A, Goss JA, Karpen S, et al. Vaccinations for adult solid-organ transplant recipients: current recommendations and protocols. *Clin Microbiol Rev*. 2003;16:357–364.
328. Mohty B, Bel M, Vukicevic M, et al. Graft-versus-host disease is the major determinant of humoral responses to the AS03-adjuvanted influenza A/09/H1N1 vaccine in allogeneic hematopoietic stem cell transplant recipients. *Haematologica*. 2011;96:896–904.
329. Salles MJ, Sens YA, Boas LS, et al. Influenza virus vaccination in kidney transplant recipients: serum antibody response to different immunosuppressive drugs. *Clin Transplant*. 2010;24:E17–E23.
330. Siegrist CA, Ambrosioni J, Bel M, et al. Responses of solid organ transplant recipients to the AS03-adjuvanted pandemic influenza vaccine. *Antivir Ther*. 2012;17:893–903.
331. Azevedo LS, Gerhard J, Miraglia JL, et al. Seroconversion of 2009 pandemic influenza A (H1N1) vaccination in kidney transplant patients and the influence of different risk factors. *Transpl Infect Dis*. 2013;15:612–618.
332. Nelson KE, Clements ML, Miotti P, et al. The influence of human immunodeficiency virus (HIV) infection on antibody responses to influenza vaccines. *Ann Intern Med*. 1988;109:383–388.
333. Kroon FP, van Dissel JT, de Jong JC, et al. Antibody response after influenza vaccination in HIV-infected individuals: a consecutive 3-year study. *Vaccine*. 2000;18:3040–3049.
334. Kubiet MA, Gonzalez-Rothi RJ, Cottey R, et al. Serum antibody response to influenza vaccine in pulmonary patients receiving corticosteroids. *Chest*. 1996;110:367–370.
335. Park CL, Frank AL, Sullivan M, et al. Influenza vaccination of children during acute asthma exacerbation and concurrent prednisone therapy. *Pediatrics*. 1996;98:196–200.
336. Beyer WEP, Palache AM, de Jong JC, et al. Cold-adapted live influenza vaccine versus inactivated vaccine: systemic vaccine reactions, local and systemic antibody response, and vaccine efficacy: a meta-analysis. *Vaccine*. 2002;20:1340–1353.
337. Boyce TG, Gruber WC, Coleman-Dockery SD, et al. Mucosal immune response to trivalent live attenuated intranasal influenza vaccine in children. *Vaccine*. 1999;18:82–88.
338. Keitel WA, Couch RB, Quarles JM, et al. Trivalent attenuated cold-adapted influenza virus vaccine: reduced viral shedding and serum antibody responses in susceptible adults. *J Infect Dis*. 1993;167:305–311.
339. Treanor JJ, Kotloff K, Betts RF, et al. Evaluation of trivalent, live, cold-adapted (CAIV-T) and inactivated (TIV) influenza vaccines in prevention of virus infection and illness following challenge of adults with wild-type influenza A (H1N1), a (H3N2), and B viruses. *Vaccine*. 1999;18:899–906.
340. Clements ML, Murphy BR. Development and persistence of local and systemic antibody responses in adults given live attenuated or inactivated influenza A virus vaccine. *J Clin Microbiol*. 1986;23:66–72.
341. Powers DC, Fries LF, Murphy BR, et al. In elderly persons live attenuated influenza A virus vaccines do not offer an advantage over inactivated virus vaccine in inducing serum or secretory antibodies or local immunologic memory. *J Clin Microbiol*. 1991;29:498–505.
342. Powers DC, Murphy BR, Fries L, et al. Reduced infectivity of cold-adapted influenza A H1N1 viruses in the elderly: correlation with serum and local antibodies. *J Am Geriatr Soc*. 1992;40:163–167.
343. He X-S, Holmes TH, Zhang C, et al. Cellular immune responses in children and adults receiving inactivated or live attenuated influenza vaccines. *J Virol*. 2006;80:11756–11766.
344. He X-S, Holmes TH, Mahmood K, et al. Phenotypic changes in Influenza-Specific CD8+ T cells after immunization of children and adults with influenza vaccines. *J Infect Dis*. 2008;197:803–811.
345. Bucasas KL, Franco LM, Shaw CA, et al. Early patterns of gene expression correlate with the humoral immune response to influenza vaccination in humans. *J Infect Dis*. 2011;Advance Access.
346. Nakaya HI, Wrammert J, Lee EK, et al. Systems biology of vaccination for seasonal influenza in humans. *Nat Immunol*. 2011;12:786–795.
347. Querec TD, Akondy RS, Lee EK, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol*. 2009;10:116–125.
348. Zhu W, Higgs BW, Morehouse C, et al. A whole genome transcriptional analysis of the early immune response induced by live attenuated and inactivated influenza vaccines in young children. *Vaccine*. 2010;28:2865–2876.
349. Petrie JG, Ohmit SE, Johnson E, et al. Efficacy studies of influenza vaccines: effect of end points used and characteristics of vaccine failures. *J Infect Dis*. 2011;203:1309–1315.
350. Edwards KM, Dupont WD, Westrich MK, et al. A randomized controlled trial of cold-adapted and inactivated vaccines for the prevention of influenza A disease. *J Infect Dis*. 1994;169:68–76.
351. Bridges CB, Thompson WW, Meltzer MI, et al. Effectiveness and cost-benefit of influenza vaccination of healthy working adults: a randomized controlled trial. *JAMA*. 2000;284:1655–1663.
352. Osterholm MT, Kelley NS, Sommer A, et al. Influenza vaccine efficacy and effectiveness: a new look at the evidence. *Lancet Infect Dis*. 2012;12:36–44.
353. Frey S, Vesikari T, Szymczakiewicz-Multanowska A, et al. Clinical efficacy of cell culture-derived and egg-derived inactivated subunit influenza vaccines in healthy adults. *Clin Infect Dis*. 2010;51:997–1004.
354. Treanor JJ, El Sahly HM, King JC Jr, et al. Protective efficacy of a trivalent recombinant hemagglutinin protein vaccine (FluBlok) against influenza in healthy adults: a randomized, placebo-controlled trial. *Vaccine*. 2011;29:7733–7739.
355. DiazGranados CA, Dunning AJ, Kimmel M, et al. Efficacy of high-dose versus standard-dose influenza vaccine in older adults. *N Engl J Med*. 2014;371:635–645.
356. Dunkle LM, Izikson R, Patriarca P, et al. Efficacy of recombinant influenza vaccine in adults 50 years of age or older. *N Engl J Med*. 2017;376:2427–2436.
357. Tasker SA, Treanor JJ, Paxton WB, et al. Efficacy of influenza vaccination in HIV-infected persons: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 1999;131:430–433.
358. Mahdi SA, Maskew M, Koen A, et al. Trivalent inactivated influenza vaccine in African adults infected with human immunodeficiency virus: double blind, randomized clinical trial of efficacy, immunogenicity, and safety. *Clin Infect Dis*. 2011;52:128–137.
359. Hoberman A, Greenberg DP, Paradise JL, et al. Effectiveness of inactivated influenza vaccine in preventing acute otitis media in young children: a randomized controlled trial. *JAMA*. 2003;290:1608–1616.
360. Neuzil KM, Dupont WD, Wright PF, et al. Efficacy of inactivated and cold-adapted vaccines against influenza A infection, 1985 to 1990: the pediatric experience. *Pediatr Infect Dis J*. 2001;20:733–740.
361. Jain VK, Rivera L, Zaman K, et al. Vaccine for prevention of mild and moderate-to-severe influenza in children. *N Engl J Med*. 2013;369:2481–2491.
362. Vesikari T, Knuf M, Wutzler P, et al. Oil-in-water emulsion adjuvant with influenza vaccine in young children. *N Engl J Med*. 2011;365:1406–1416.
363. Vesikari T, Kirstein J, Devota Go G, et al. Efficacy, immunogenicity, and safety evaluation of an MF59-adjuvanted quadrivalent influenza virus vaccine compared with non-adjuvanted influenza vaccine in children: a multicentre, randomised controlled, observer-blinded, phase 3 trial. *Lancet Respir Med*. 2018.
364. Belshe RB, Mendelman PM, Treanor J, et al. The efficacy of live attenuated cold-adapted trivalent, intranasal influenza virus vaccine in children. *N Engl J Med*. 1998;358:1405–1412.
365. Belshe RB, Gruber WC, Mendelman PM, et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. *J Pediatr*. 2000;136:168–175.
366. Vesikari T, Fleming DM, Aristegui JF, et al. Safety, efficacy, and effectiveness of Cold-Adapted influenza Vaccine-Trivalent against Community-Acquired, Culture-Confirmed influenza in young children attending day care. 10.1542/peds.2006-0725. *Pediatrics*. 2006;118:2298–2312.
367. Tam JS, Capeding MR, Lum LC, et al. Efficacy and safety of a live attenuated, cold-adapted influenza vaccine, trivalent against culture-confirmed influenza in young children in Asia. *Pediatr Infect Dis J*. 2007;26:619–628.
368. Nichol KL, Mendelman PM, Mallon KP, et al. Effectiveness of live, attenuated intranasal influenza virus vaccine in healthy, working adults: a randomized controlled trial. *JAMA*. 1999;282:137–144.
369. DeVilliers PJT, Steele AD, Hiemstra LA, et al. Efficacy and safety of a live attenuated influenza vaccine in adults 60 years of age and older. *Vaccine*. 2010;28:228–234.
370. Orenstein EW, de Serres G, Haber MJ, et al. Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. *Int J Epidemiol*. 2007;36:623–631.
371. Treanor JJ, Talbot HK, Ohmit SE, et al. Effectiveness of seasonal influenza vaccine in the United States during a season with circulation of all three vaccine strains. *Clin Infect Dis*. 2012;55:951–959.
372. Skowronski DM, Janjua NZ, de Serres G, et al. A sentinel platform to evaluate influenza vaccine effectiveness and new variant circulation, Canada 2010-2011 season. *Clin Infect Dis*. 2012;55:332–342.
373. Talbot HK, Griffin MR, Chen Q, et al. Effectiveness of seasonal vaccine in preventing confirmed influenza-associated hospitalizations in community dwelling older adults. *J Infect Dis*. 2011.
374. Arriola C, Garg S, Anderson EJ, et al. Influenza vaccination modifies disease severity among Community-dwelling adults hospitalized with influenza. *Clin Infect Dis*. 2017;65:1289–1297.
375. Shay DK, Chillarige Y, Kelman J, et al. Comparative effectiveness of high-dose versus standard-dose influenza vaccines among US Medicare beneficiaries in preventing postinfluenza deaths during 2012-2013 and 2013-2014. *J Infect Dis*. 2017;215:510–517.
376. Van Buynder PG, Konrad S, Van Buynder JL, et al. The comparative effectiveness of adjuvanted and unadjuvanted trivalent inactivated influenza vaccine (TIV) in the elderly. *Vaccine*. 2013;31:6122–6128.
377. Ferdinands JM, Shay DK. Magnitude of potential biases in a simulated case-control study of the effectiveness of influenza vaccination. *Clin Infect Dis*. 2012;54:25–32.
378. Kelly H, Jacoby P, Dixon G, et al. Vaccine effectiveness against laboratory-confirmed influenza in healthy young children. *Pediatr Infect Dis J*. 2011;30:107–111.

379. Skowronski DM, Chambers C, Sabaiduc S, et al. A perfect storm: impact of genomic variation and serial vaccination on low influenza vaccine effectiveness during the 2014–2015 Season. *Clin Infect Dis*. 2016;63:21–32.
380. Fonville JM, Wilks SH, James SL, et al. Antibody landscapes after influenza virus infection or vaccination. *Science*. 2014;346:996–1000.
381. Ashkenazi S, Vertruyen A, Aristegui J, et al. Superior relative efficacy of live attenuated influenza vaccine compared with inactivated influenza vaccine in young children with recurrent respiratory tract infections. *Pediatr Infect Dis J*. 2006;25:870–879.
382. Ohmit SE, Victor JC, Rotthoff JR, et al. Prevention of antigenically drifted influenza by inactivated and live attenuated vaccines. *N Engl J Med*. 2006;355:2513–2522.
383. Monto AS, Ohmit SE, Petrie JG, et al. Comparative efficacy of inactivated and live attenuated influenza vaccines. *N Engl J Med*. 2009;361:1260–1267.
384. Wang Z, Tobler S, Roayaei J, et al. Live attenuated or inactivated influenza vaccines and medical encounters for respiratory illnesses among US military personnel. *JAMA*. 2009;301:945–953.
385. Chung JR, Flannery B, Thompson MG, et al. Seasonal effectiveness of live attenuated and inactivated influenza vaccine. *Pediatrics*. 2016;137:1–10.
386. Monto AS, Davenport FM, Napier JA, et al. Modification of an outbreak of influenza in tecumseh, Michigan by vaccination of schoolchildren. *J Infect Dis*. 1970;122:16–25.
387. Loeb M, Russell ML, Moss L, et al. Effect of influenza vaccination of children on infection rates in hutterite communities: a randomized trial. *JAMA*. 2010;303:943–950.
388. Hurwitz ES, Haber M, Chang A, et al. Effectiveness of influenza vaccination of day care children in reducing influenza-related morbidity among household contacts. *JAMA*. 2000;284:1677–1682.
389. Reichert TA, Sugaya N, Fedson DS, et al. The Japanese experience with vaccinating schoolchildren against influenza. *N Engl J Med*. 2001;344:889–896.
390. Kawai S, Nanri S, Ban E, et al. Influenza vaccination of schoolchildren and influenza outbreaks in a school. *Clin Infect Dis*. 2011;53:130–136.
391. CDC. Influenza vaccination of health care personnel. *MMWR Morb Mortal Wkly Rep*. 2006;55:1–12.
392. Wilde JA, McMillan JA, Serwint J, et al. Effectiveness of influenza vaccine in health care professionals: a randomized trial. *JAMA*. 1999;281:908–913.
393. Salgado CD, Gianetta ET, Hayden FG, et al. Preventing nosocomial influenza by improving the vaccine acceptance rate of clinicians. *Infect Control Hosp Epidemiol*. 2004;25:923–928.
394. Potter J, Stott DJ, Roberts MA, et al. Influenza vaccination of health care workers in long-term-care hospitals reduces the mortality of elderly patients. *J Infect Dis*. 1997;175:1–6.
395. Carman WF, Elder AG, Wallace LA, et al. Effects of influenza vaccination of health-care workers on mortality of elderly people in long-term care: a randomised controlled trial [see comments]. *Lancet*. 2000;355:93–97.
396. Zaman K, Roy E, Arifeen SE, et al. Effectiveness of maternal influenza immunization in mothers and infants. *N Engl J Med*. 2008;359:1555–1564.
397. Tapia MD, Sow SO, Tamboura B, et al. Maternal immunisation with trivalent inactivated influenza vaccine for prevention of influenza in infants in Mali: a prospective, active-controlled, observer-blind, randomised phase 4 trial. *Lancet Infect Dis*. 2016;16:1026–1035.
398. Mahdi SA, Nunes MC, Cutland CL. Influenza vaccination of pregnant women and protection of their infants. *N Engl J Med*. 2014;371:2340.
399. Benowitz I, Esposito DB, Gracey KD, et al. Influenza vaccine given to pregnant women reduces hospitalization due to influenza in their infants. *Clin Infect Dis*. 2010;51:1355–1361.
400. Treanor JJ, Campbell JD, Zangwill KM, et al. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. *N Engl J Med*. 2006;354:1343–1351.
401. Treanor JJ, Wilkinson BE, Masseoud F, et al. Safety and immunogenicity of a recombinant hemagglutinin vaccine for H5 influenza in humans. *Vaccine*. 2001;19:1732–1737.
402. Bresson J-L, Perronne C, Launay O, et al. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomized trial. *Lancet*. 2006;367:1657–1664.
403. Belshe RB, Frey SE, Graham I, et al. Safety and immunogenicity of influenza A H5 subunit vaccines: effect of vaccine schedule and antigenic variant. *J Infect Dis*. 2011;203:666–673.
404. Lin J, Zhang J, Dong X, et al. Safety and immunogenicity of an inactivated adjuvanted whole-virion influenza A (H5N1) vaccine: a phase I randomised controlled trial. *Lancet*. 2006;368:991–997.
405. Atmar RL, Keitel WA, Patel SM, et al. Safety and immunogenicity of nonadjuvanted and MF59-adjuvanted influenza A/H9N2 vaccine preparations. *Clin Infect Dis*. 2006;43:1135–1142.
406. Leroux-Roels I, Borkowski A, Vanwolleghem T, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rh5n1 prototype pandemic influenza vaccine: a randomised controlled trial. *Lancet*. 2007;370:580–589.
407. Galli G, Hancock K, Hoschler K, et al. Fast rise of broadly cross-reactive antibodies after boosting long-lived human memory B cells primed by an MF59 adjuvanted prepandemic vaccine. *Proc Nat Acad Sci*. 2009;106:7962–7967.
408. Khurana S, Verma N, Yewdell JW, et al. MF59 adjuvant enhances diversity and affinity of antibody-mediated immune response to pandemic influenza vaccines. *Sci Transl Med*. 2011;3:85ra48.
409. Jackson LA, Campbell JD, Frey SE, et al. Effect of varying doses of a monovalent H7N9 influenza vaccine with and without AS03 and MF59 adjuvants on immune response: a randomized clinical trial. *JAMA*. 2015;314:237–246.
410. Stephenson I, Nicholson KG, Colegate AE, et al. Boosting immunity to influenza H5N1 with MF59-adjuvanted h5n3 A/Duck/Singapore/97 vaccine in a primed human population. *Vaccine*. 2003;21:1687–1693.
411. Goji NA, Nolan C, Hill H, et al. Immune responses of healthy subjects to a single dose of intramuscular inactivated influenza A/Vietnam/1203/04 H5N1 vaccine after priming with an antigenic variant. *J Infect Dis*. 2008;198:635–641.
412. Karron R, Callahan K, Luke C, et al Phase I evaluation of live attenuated H9N2 and H5N1 ca reassortant vaccines in healthy adults. Third WHO meeting on evaluation of pandemic influenza prototype vaccines in clinical trials, 15-16 February 2007; 2007; Geneva, Switzerland.
413. Karron RA Clinical evaluation of live attenuated pandemic influenza virus vaccines. Fourth WHO meeting on evaluation of pandemic influenza prototype vaccines in clinical trials, 14-15 February 2008; 2008; Geneva, Switzerland.
414. Talaat KR, Luke CJ, Khurana S, et al. A live attenuated influenza A (H5N1) vaccine induces long-term immunity in the absence of a primary antibody response. *J Infect Dis*. 2014;209:1860–1869.
415. Babu T, Levine M, Fitzgerald T, et al. Live attenuated H7N7 influenza vaccine primes for a vigorous antibody response to inactivated H7N7 influenza vaccine. *Vaccine*. 2014;32:6798–6804.
416. Sobhanie M, Matsuoka Y, Jegaskanda S, et al. Evaluation of the safety and immunogenicity of a candidate pandemic live attenuated influenza vaccine (pLAIV) against influenza A(H7N9). *J Infect Dis*. 2016;213:922–929.
417. Goodman JL. Investing in immunity: Pre-pandemic immunization to combat future influenza pandemics. *Clin Infect Dis*. 2015;IN PRESS.
418. Krammer F, Palese P. Influenza virus hemagglutinin stalk-based antibodies and vaccines. *Curr Opin Virol*. 2013;3:521–530.
419. Krammer F, Pica N, Hai R, et al. Chimeric hemagglutinin influenza virus vaccine constructs elicit broadly protective stalk-specific antibodies. *J Virol*. 2013;87:6542–6550.
420. Krammer F, Margine I, Hai R, et al. H3 stalk-based chimeric hemagglutinin influenza virus constructs protect mice from H7N9 challenge. *J Virol*. 2014;88:2340–2343.
421. Steel J, Lowen AC, Wang TT, et al. Influenza virus vaccine based on the conserved hemagglutinin stalk domain. *MBio*. 2010;1:pii: e00018-10.
422. Wang TT, Tan GS, Hai R, et al. Vaccination with a synthetic peptide from the influenza virus hemagglutinin provides protection against distinct viral subtypes. *Proc Natl Acad Sci USA*. 2010;107:18979–18984.
423. Lu Y, Welsh JP, Swartz JR. Production and stabilization of the trimeric influenza hemagglutinin stem domain for potentially broadly protective influenza vaccines. *Proc Natl Acad Sci USA*. 2014;111:125–130.
424. Sandbulte MR, Westgeest KB, Gao J, et al. Discordant antigenic drift of neuraminidase and hemagglutinin in H1N1 and H3N2 influenza viruses. *Proc Natl Acad Sci USA*. 2011;108:20748–20753.
425. Fiers W, De Filette M, El Bakkouri K, et al. M2e-based universal influenza A vaccine. *Vaccine*. 2009;27:6280–6283.
426. Atsmon J, Caraco Y, Ziv-Sefer S, et al. Priming by a novel universal influenza vaccine (Multimeric-001)-a gateway for improving immune response in the elderly population. *Vaccine*. 2014;32:5816–5823.
427. Lillie PJ, Berthoud TK, Powell TJ, et al. Preliminary assessment of the efficacy of a T-Cell–Based Influenza vaccine, MVA-NP+M1, in humans. *Clin Infect Dis*. 2012;55:19–25.
428. Kwon JS, Yoon J, Kim YJ, et al. Vaccinia-based influenza vaccine overcomes previously induced immunodominance hierarchy for heterosubtypic protection. *Eur J Immunol*. 2014;44:2360–2369.
429. Hemann EA, Kang SM, Legge KL. Protective CD8 T cell-mediated immunity against influenza A virus infection following influenza virus-like particle vaccination. *J Immunol*. 2013;191:2486–2494.
430. Furuya Y. Return of inactivated whole-virus vaccine for superior efficacy. *Immunol Cell Biol*. 2012;90:571–578.
431. Bui M, Whittaker G, Helenius A. Effect of M1 protein and low pH on nuclear transport of influenza virus ribonucleoproteins. *J Virol*. 1996;70:8391–8401.
432. Dolin R, Reichman RC, Madore HP, et al. A controlled trial of amantadine and rimantadine in the prophylaxis of influenza A in humans. *N Engl J Med*. 1982;307:580–584.
433. Van Voris LP, Betts RF, Hayden FG, et al. Successful treatment of naturally occurring influenza A/USSR/77 H1N1. *J Am Med Assoc*. 1981;245:1128–1131.
434. Hayden FG, Monto AS. Oral rimantadine hydrochloride therapy of influenza A virus H3N2 subtype infection in adults. *Antimicrob Agents Chemother*. 1986;29:339–341.
435. Hall CB, Dolin R, Gala CL, et al. Children with influenza A infection: treatment with rimantadine. *Pediatrics*. 1987;80:275–282.
436. Thompson J, Fleet W, Lawrence E, et al. A comparison of acetaminophen and rimantadine in the treatment of influenza A infection in children. *J Med Virol*. 1987;21:249–255.
437. Shiraishi K, Mitamura K, Sakai Y, et al. High frequency of resistant viruses harboring different mutations in amantadine-treated children with influenza. *J Infect Dis*. 2003;188.
438. Hay AJ, Wolstenholme AJ, Skehel JJ, et al. The molecular basis of the specific anti-influenza action of amantadine. *EMBO J*. 1985;4:3021–3024.
439. Boivin G, Goyette N, Bernatchez H. Prolonged excretion of amantadine-resistant influenza A virus quasi species after cessation of antiviral therapy in an immunocompromised patient. *Clin Infect Dis*. 2002;34:E23–E25.
440. Bright RA, Medina M-J, Xu X, et al. Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. *Lancet*. 2005;366:1175–1181.
441. Bright RA, Shay DK, Shu B, et al. Adamantane resistance among influenza A viruses isolated early during the 2005-2006 influenza season in the United States. *JAMA*. 2006;295:891–894.
442. Mitnaul LJ, Castrucci MR, Murti KG, et al. The cytoplasmic tail of influenza A virus neuraminidase (NA) affects NA incorporation into virions, virion morphology, and virulence in mice but is not essential for virus replication. *J Virol*. 1996;70:873–879.
443. Matrosovich MN, Matrosovich TY, Gray T, et al. Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. *J Virol*. 2004;78:12665–12667.
444. Barroso L, Treanor J, Gubareva L, et al. Efficacy and tolerability of the oral neuraminidase inhibitor peramivir in experimental human influenza: randomized, controlled trials for prophylaxis and treatment. *Antivir Ther*. 2005;10:901–910.
445. Alame MM, Massaad E, Zaraket H. Peramivir: a novel intravenous neuraminidase inhibitor for treatment of acute influenza infections. *Front Microbiol*. 2016;7:450.
446. Treanor JJ, Hayden FG, Vrooman PS, et al. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized, controlled trial. *J Am Med Assoc*. 2000;283:1016–1024.
447. Nicholson KG, Aoki FY, Osterhaus ADME, et al. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomized controlled trial. *Lancet*. 2000;355:1845–1850.
448. Hayden FG, Osterhaus ADME, Treanor JJ, et al. Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infections. *N Engl J Med*. 1997;337:874–880.
449. MIST. Randomised trial of efficacy and safety of inhaled zanamivir in treatment of influenza A and B virus infections. *Lancet*. 1998;352:1877–1881.
450. Kohno S, Kida H, Mizuguchi M, et al. Efficacy and safety of intravenous peramivir for treatment of seasonal influenza virus infection. *Antimicrob Agents Chemother*. 2010;54:4568–4574.
451. de Jong MD, Ison MG, Monto AS, et al. Evaluation of intravenous peramivir for treatment of influenza in hospitalized patients. *Clin Infect Dis*. 2014;59:e172–e185.
452. Whitley RJ, Hayden FG, Reisinger KS, et al. Oral oseltamivir treatment of influenza in children. *Pediatr Infect Dis J*. 2001;20:127–133.
453. Hedrick JA, Barzilai A, Behre U, et al. Zanamivir for treatment of symptomatic influenza A and B infection in

children five to twelve years of age: a randomized controlled trial. *Pediatr Infect Dis J*. 2000;19:410–417.
454. McClellan K, Perry CM. Oseltamivir: a review of its use in influenza. *Drugs*. 2001;61:263–283.
455. Lalezari J, Campion K, Keene O, et al. Zanamivir for the treatment of influenza A and B infection in high-risk patients: a pooled analysis of randomized controlled trials. *Arch Intern Med*. 2001;161:212–217.
456. Kaiser L, Wat C, Mills T, et al. Impact of oseltamivir treatment on influenza-related lower respiratory tract complications and hospitalizations. *Arch Intern Med*. 2003;163:1667–1672.
457. Hernan MA, Lipsitch M. Oseltamivir and risk of lower respiratory tract complications in patients with flu symptoms: a meta-analysis of eleven randomized clinical trials. *Clin Infect Dis*. 2011;53:277–279.
458. Hiba V, Chowers M, Levi-Vinograd I, et al. Benefit of early treatment with oseltamivir in hospitalized patients with documented 2009 influenza A (H1N1): retrospective cohort study. *J Antimicrob Chemother*. 2011;66:1150–1155.
459. Rodriguez A, Dia E, Martin-Loeches I, et al. Impact of early oseltamivir treatment on outcome in critically ill patients with 2009 pandemic influenza a. *J Antimicrob Chemother*. 2011;66.
460. Louie JK, Yang S, Acosta M, et al. Treatment with neuraminidase inhibitors for critically ill patients with influenza A (H1N1)pdm09. *Clin Infect Dis*. 2012;55:1198–1204.
461. Piedra PA, Schulman KL, Blumentals WA. Effects of oseltamivir on influenza-related complications in children with chronic medical conditions. *Pediatrics*. 2009;124:170–178.
462. Gubareva LV, Bethell R, Hart GJ, et al. Characterization of mutants of influenza A selected with the neuraminidase inhibitor 4-guanidino-Neu5Ac2en. *J Virol*. 1996;70:1818–1827.
463. Gubareva LV, Robinson MJ, Bethell RC, et al. Catalytic and framework mutations in the neuraminidase active site of influenza viruses that are resistant to 4-guanidino-neu5ac2en. *J Virol*. 1997;71:3385–3390.
464. Moscona A. Oseltamivir resistance - disabling our influenza defenses. *N Engl J Med*. 2005;353:2633–2636.
465. Blick TJ, Sahasrabudhe A, McDonald M, et al. The interaction of neuraminidase and hemagglutinin mutations in influenza virus in resistance to 4-guanidino-neu5ac2en. *Virology*. 1998;246:95–103.
466. Kiso M, Mitamura K, Sakai-Tagawa Y, et al. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. *Lancet*. 2004;364:759–765.
467. Ives JA, Carr JA, Mendel DB, et al. The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leave virus severely compromised both in vitro and in vivo. *Antiviral Res*. 2002;55:307–317.
468. Carr J, Ives J, Kelly L, et al. Influenza virus carrying neuraminidase with reduced sensitivity to oseltamivir carboxylate has altered properties in vitro and is compromised for infectivity and replicative ability in vivo. *Antiviral Res*. 2002;54:79–88.
469. Herlocher ML, Carr J, Ives J, et al. Influenza virus carrying an R292K mutation in the neuraminidase gene is not transmitted in ferrets. *Antiviral Res*. 2002;54:99–111.
470. Herlocher ML, Truscon R, Elias S, et al. Influenza viruses resistant to the antiviral drug oseltamivir: transmission studies in ferrets. *J Infect Dis*. 2004;190:1627–1630.
471. Seibert CW, Rhahmat S, Krammer F, et al. Efficient transmission of pandemic H1N1 influenza viruses with high-level oseltamivir resistance. *J Virol*. 2012;86:5386–5389.
472. Dharan JN, Gubareva LV, Meyer JJ, et al. Infections with oseltamivir-resistant influenza A(H1N1) virus in the United States. *JAMA*. 2009;301:1034–1041.
473. Whitley RJ, Boucher CA, Lina B, et al. Global assessment of resistance to neuraminidase inhibitors, 2008-2011: The influenza resistance information study (IRIS). *Clin Infect Dis*. 2013;56:1197–1205.
474. Hurt AC, Hardie K, Wilson NJ, et al. Characteristics of a widespread community cluster of h2758y oseltamivir-resistant a(H1N1)pdm09 influenza in Australia. *J Infect Dis*. 2012;206:148–157.
475. Trevejo JM, Asmal M, Vingerhoets J, et al. Pimodivir treatment in adult volunteers experimentally inoculated with live influenza virus: a phase IIa, randomized, double-blind, placebo-controlled study. *Antivir Ther*. 2018;23:335–344.
476. McKimm-Breschkin JL, Jiang S, Hui DS, et al. Prevention and treatment of respiratory viral infections: presentations on antivirals, traditional therapies and host-directed interventions at the 5th ISIRV antiviral group conference. *Antiviral Res*. 2018;149:118–142.
476a. US Food and Drug Administration. FDA approves new drug to treat influenza. FDA News Release; October 24, 2018. https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm624226.htm. Accessed February 20, 2019.
476b. Hayden FG, Sugaya N, Hirotsu N, et al. Baloxavir Marboxil for Uncomplicated Influenza in Adults and Adolescents. *N Engl J Med*. 2018;379:913–923.
477. Yamashita M. Laninamivir and its prodrug CS-8958: long-acting neuraminidase inhibitors for the treatment of influenza. *Antivir Chem Chemother*. 2010;21:71–84.
478. Furuta Y, Takahashi K, Kuno-Maekawa M, et al. Mechanism of action of T-705 against influenza virus. 10.1128/AAC.49.3.981-986.2005. *Antimicrob Agents Chemother*. 2005;49:981–986.
479. Haffizulla J, Hartman A, Hoppers M, et al. Effect of nitazoxanide in adults and adolescents with acute uncomplicated influenza: a double-blind, randomised, placebo-controlled, phase 2b/3 trial. *Lancet Infect Dis*. 2014;14:609–618.
480. Nguyen JT, Smee DF, Barnard DL, et al. Efficacy of combined therapy with amantadine, oseltamivir, and ribavirin in vivo against susceptible and amantadine-resistant influenza A viruses. *PLoS ONE*. 2012;7:e31006.
481. Kim W-Y, Young Suh G, Huh JW, et al. Triple-Combination antiviral drug for pandemic H1N1 influenza virus infection in critically ill patients on mechanical ventilation. *Antimicrob Agents Chemother*. 2011;55:5703–5709.
482. Beigel JH, Bao Y, Beeler J, et al. Oseltamivir, amantadine, and ribavirin combination antiviral therapy versus oseltamivir monotherapy for the treatment of influenza: a multicentre, double-blind, randomised phase 2 trial. *Lancet Infect Dis*. 2017;17:1255–1265.
483. Muthuri SG, Venkatesan S, Myeles P, et al. Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted t hospital with influenza A h1n1pdm09 virus infection: a meta-analysis of individual participant data. *Lancet Respir Med*. 2014;2:395–404.
484. Monto AS, Robinson DP, Herlocher ML, et al. Zanamivir in the prevention of influenza among healthy adults: a randomized controlled trial. *JAMA*. 1999;282:31–35.
485. Hayden FG, Atmar RL, Schilling M, et al. Use of the selective oral neuraminidase inhibitor oseltamivir to prevent influenza. *N Engl J Med*. 1999;341:1336–1346.
486. Peters PH Jr, Gravenstein S, Norwood P, et al. Long-term use of oseltamivir for the prophylaxis of influenza in a vaccinated frail older population. *J Am Geriatr Soc*. 2001;49:1025–1031.
487. Welliver R, Monto AS, Carewicz O, et al. Effectiveness of oseltamivir in preventing influenza in household contacts: a randomized controlled trial. *J Am Med Assoc*. 2001;285:748–754.
488. Hayden FG, Gubareva LV, Monto AS, et al. Inhaled zanamivir for the prevention of influenza in families. *N Engl J Med*. 2000;343:1282–1289.
489. Mast EE, Harman MW, Gravenstein S, et al. Emergence and possible transmission of amantadine-resistant viruses during nursing home outbreaks of influenza A(H3N2). *Am J Epidemiol*. 1991;134:988–997.
490. Degelau J, Somani SK, Cooper SL, et al. Amantadine-resistant influenza A in a nursing facility. *Arch Intern Med*. 1992;152:390–392.

166 California Encephalitis, Hantavirus Pulmonary Syndrome, Hantavirus Hemorrhagic Fever With Renal Syndrome, and Bunyavirus Hemorrhagic Fevers

Raphael Dolin

SHORT VIEW SUMMARY

Definition
- Bunyavirales is a large order of RNA viruses consisting of 10 families and more than 350 named species. They are enveloped, single-stranded RNA viruses with a segmented genome. Bunyavirales members can be found worldwide and are able to infect invertebrates, vertebrates, and plants.

Epidemiology
- Bunyaviruses are significant human pathogens with the ability to cause severe disease, ranging from febrile illness, encephalitis, and hepatitis to hemorrhagic fever.
- With exception of hantaviruses, bunyaviruses are transmitted by hematophagous arthropods, including ticks, mosquitoes, and phlebotomine flies.
- Hantaviruses are maintained in nature through persistent infection of rodents.

Major Causes of Human Diseases (See Table 166.1)
- California encephalitis group:
  - La Crosse virus (LACV)
  - Jamestown Canyon virus (JCV)
- Rift Valley fever virus (RVFV)
- Crimean-Congo hemorrhagic fever virus (CCHFV)
- Hantaviruses
  - Hemorrhagic fever with renal syndrome (HFRS)
  - Hantavirus pulmonary syndrome
- Severe fever with thrombocytopenia syndrome virus (SFTSV)

Diagnosis
- In most cases the diagnosis is based on serologic investigation of acute and early convalescent sera. Early diagnosis can be based on reverse-transcriptase polymerase chain reaction assays of blood samples. Diagnostic tests are typically performed in reference laboratories.

Therapy
- Treatment is primarily supportive because specific antiviral therapy is not available. Ribavirin has been studied in some bunyavirus infections, and data from in vitro and in vivo models hold promise. Ribavirin has shown clinical benefit in HFRS and in CCHF. However, comprehensive clinical trials have not been conducted.

Prevention
- No specific preventive measures are available, but experimental vaccines for some bunyaviruses have been developed and are undergoing study. Individual protective measures, such as arthropod repellents and insecticide-impregnated mosquito bed nets, are recommended in endemic areas.

Bunyaviruses are an extraordinarily diverse and numerous group of viruses whose taxonomic complexity has led to a recently reorganized classification by the International Committee on Taxonomy of Viruses (ICTV). In 2017 the ICTV reclassified the family Bunyaviridae to that of an order, Bunyavirales, that comprises 10 families: Arenaviridae, Cruliviridae, Fimoviridae, Hantaviridae, Mypoviridae, Nairoviridae, Peribunyaviridae, Phasmaviridae, Phenuiviridae, and Wupedeviridae. The families comprise 35 genera, with more than 350 named viruses identified as species.[1,2] Classification into separate families and species is based on genomic, structural, biochemical, and antigenic chracteristics,[3] although limited data are available for a number of these viruses. The distribution of these viruses is via arthropod-vertebrate cycles or by chronic infection of vertebrates, which lead to epidemiologic patterns that are ecologically determined and can be focal or diffuse depending on climatic variables. Table 166.1 presents salient features of infections caused by Bunyavirales viruses, including vectors, transmission, epidemiology, and clinical expression. Details of these infections are discussed further as follows.

VIROLOGY

Structure, Replication, and Antigenic Relationships

Bunyavirales members are spherical, enveloped RNA viruses, 80 to 120 nm in diameter and that display surface glycoproteins of 5 to 10 nm.[3] The RNA is of negative sense or ambisense, and codes for six or fewer proteins. The genome consists of two to six segments, although the majority of bunyaviruses studied have a trisegmented genome (Fig. 166.1).[3–5] The large RNA segment (L) encodes an RNA-dependent RNA polymerase; the medium segment (M) encodes viral glycoprotein precursors, which are cleaved by the host protease to yield mature glycoproteins, Gn and Gc, that are involved in receptor binding and endomembrane fusion. The small genome segment (S) encodes the nucleocapsid proteins.[3–5] The molecular weights of the proteins and RNAs vary by genus. In general, the Gn or Gc proteins, or both, are targets for neutralizing antibodies.[3] The nucleocapsid protein is believed to be the most important source of immunologic relationships observed within and across families and genera. In general, the fluorescent antibody test is the most cross-reactive, with hemagglutination inhibition

(HI), and although the neutralization tests provide greater specificity, the latter is of greatest use in distinguishing individual viruses. Enzyme-linked immunosorbent assays (ELISAs) increasingly are used for diagnosis of acute or resolving (immunoglobulin [Ig]M) or retrospective (IgG) infections (see later).

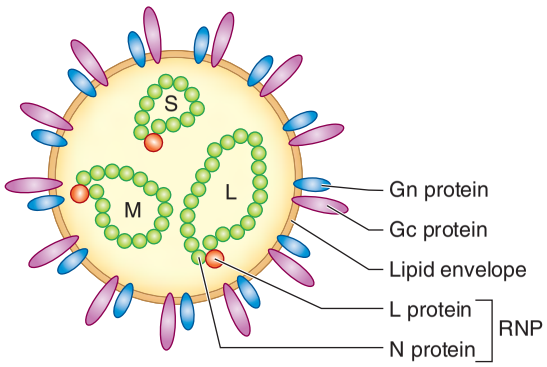

FIG. 166.1 Bunyavirus structure. The three genome segments (*L, M,* and *S*) are encapsidated by nucleocapsid protein to form ribonucleoprotein complexes *(RNPs),* and together with the viral L protein (RNA-dependent RNA polymerase) are packaged within a host cell-derived lipid envelope modified by insertion of the viral glycoproteins *Gn* and *Gc. (Modified from Elliot RM, Schmaljohn CS. Bunyaviridae. In: Knipe DM, Howley PM, eds.* Fields Virology. *Philadelphia: Lippincott Williams & Wilkins; 2013:1245.)*

Virus replication occurs in the cytoplasm, and virions mature by budding from the Golgi complex and endoplasmic reticulum into vesicles. Some bunyaviruses are exceptions, such as Rift Valley fever virus (RVFV), which buds through the outer cell membrane of hepatocytes, and Sin Nombre virus (SNV), which matures at the cytoplasmic membrane.[3]

EPIDEMIOLOGY

Ecology and Distribution

With the exception of hantaviruses, all bunyaviruses are transmitted by arthropods (mosquitoes, ticks, and sand flies). Hantaviruses are transmitted through feces from rodent species, such as deer mice or the striped field mouse.

California Encephalitis Viruses

Among the California encephalitis (CE) virus group, La Crosse virus (LACV) is the most medically significant virus, causing an average of 79 cases per year, predominantly in children younger than 15 years.[6] Its principal vector is *Aedes triseriatus,* a forest-dwelling, tree-hole–breeding mosquito of the north-central and northeastern regions of the country. LACV is maintained in this mosquito via transovarial transmission supplemented by intraspecific venereal transmission and amplification during summer by mosquitoes feeding on viremic chipmunks, squirrels, foxes, and woodchucks.[7,8]

Female mosquitoes infected by any of these mechanisms are capable of transmitting virus via a bite. The virus survives during the winter in mosquito eggs.[9] LACV and human encephalitis were first recognized in the upper Mississippi and Ohio River valleys. Most cases have

TABLE 166.1 Some Characteristics of Severe Diseases Caused by Bunyavirales

DISEASE	GENUS AND VIRUSES	VECTOR	TRANSMISSION TO HUMANS	DISEASE PATTERN AND ANNUAL INCIDENCE	MAJOR CLINICAL FEATURES
California encephalitis	*Orthobunyavirus* La Crosse Jamestown Canyon	*Aedes triseriatus* Transovarial transmission; amplification by chipmunks	Mosquito bite	Summer-fall; northern United States: 60–130 cases	Meningoencephalitis, seizures, cerebral edema
Rift Valley fever	*Phlebovirus* Rift Valley fever	*Aedes mcintoshi* Transovarial transmission; horizontal transmission in other arthropods	Mosquito bite; aerosol or contact with fresh carcasses, domestic animals	Endemic in rainy season sub-Saharan Africa: hundreds of cases; occasional epidemics associated with exceptional rainfall	Acute febrile illness with occasional retinitis, hemorrhagic fever, or encephalitis
Crimean-Congo hemorrhagic fever	*Orthonairovirus* Crimean-Congo Hemorrhagic Fever	*Hyalomma* ticks Amplified by hares, domestic animals	Tick bite, contact with blood of humans or domestic animals	Spring-summer; regions of former Soviet Union, Middle East, Africa: 50–200 cases	Severe hemorrhagic fever
Hemorrhagic fever with renal syndrome	*Orthohantavirus* Hantaan Dobrava Seoul Puumala	Chronic infection of striped field mouse, yellow-necked mouse, rat, or bank vole	Aerosols from rodent excreta	Endemic and epidemic; season depends on local conditions. Asia, Europe: 100,000 cases	Fever, shock, bleeding, renal failure
Hantavirus pulmonary syndrome	*Orthohantavirus* Sin Nombre Bayou Black Creek Canal	Chronic infection of deer mouse and other rodents	Aerosols from rodent excreta	Discovered in 1993; dozens of cases annually in North and South America	Fever, shock, pulmonary edema
Tropical Fever	*Orthobunyavirus* Oropouche	Biting midge *Culicoides paraensis*	By biting midges	Epidemic and sporadic disease in tropical areas of Brazil, Peru, and Panama	Acute febrile illness with occasional hemorrhagic complications
Sand fly fever	*Phlebovirus* Toscana Sand fly fever	*Phlebotomus perniciosus, Phlebotomus papatasi*	Mosquito bite	Circum-Mediterranean distribution in Southern Europe	Fever, headache, myalgia, meningitis
Severe fever with thrombocytopenia syndrome (SFTS)	*Phlebovirus* SFTS	*Haemaphysalis longicornis, Ixodes nipponensis, Amblyomma testudinarium*	Tick bites, close person-to-person contact	Central and eastern China, South Korea, western Japan	Fever, multiorgan dysfunction, bleeding diathesis
Heartland virus disease	*Phlebovirus* Heartland	Tick-borne Lone Star tick *Amblyomma americanum*	Probably tick-borne	Midwestern and southern United States: 30 cases since 2012	Fever, headache, cough, confusion, myalgia, multiorgan dissemination, thrombocytopenia

been reported from Wisconsin, Minnesota, Iowa, Indiana, Ohio, and Illinois.[10,11] However, recognition of the disease in West Virginia and Georgia[10] has led to an understanding that viral transmission occurs throughout the eastern United States; during 2003–07 West Virginia had the greatest number of cases (95) in the United States.[12] Studies in Tennessee suggest recent extension into that state.[11] Other vectors are not of major importance except focally. The Asian mosquito *Aedes albopictus* is an efficient vector and is capable of horizontal and vertical transmission in the laboratory.[13] Its strongly anthropophilic biting habits and its documented extension into areas where LACV is endemic raise concern, particularly because the virus has been isolated from field collections of *A. albopictus*.[14]

Other CE viruses have distinct ecologic cycles based on an element of transovarial transmission in mosquitoes, and human disease is uncommon and usually, but not always, mild.[15,16]

Rift Valley Fever

RVFV is maintained in sub-Saharan Africa via transovarial transmission in certain floodwater-breeding *Aedes* mosquitoes, notably *Aedes mcintoshi*.[17] Infected eggs can remain dormant but viable in soil for years while awaiting heavy rains for subsequent hatching. Other mosquitoes are important during epizootics and epidemics; large domestic ungulates, such as sheep or cattle, serve as amplifiers because they experience high viremia during infection.[18] In 1977 the virus was introduced into Egypt, producing widespread epidemic disease in humans and domestic animals; it reappeared in the 1990s. After an extensive epidemic in Kenya in 1997–98, it was introduced into the Arabian peninsula, where it also caused epidemic disease in animals and humans.[19,20] Heavy rainfall in East Africa resulted in a major recurring epidemic in 2006–07.[21] It is likely that other receptive areas, such as North America, might experience the same fate if an introduction should occur.[22]

Crimean-Congo Hemorrhagic Fever

Crimean-Congo hemorrhagic fever virus (CCHFV) is transmitted by ticks. It was first described in Russian soldiers in the Crimea in 1944 and named Crimean fever. Subsequently, an identical virus was isolated from an ill child in the Congo in 1956 and was named Congo virus. After the viruses were shown to be identical, they were renamed Crimean-Congo hemorrhagic fever virus.[23–25] The principal vectors belong to the genus *Hyalomma*. Immature stages feed on hares, hedgehogs, and ground-feeding birds, whereas adults parasitize large wild and domestic animals. This virus is widely distributed in southwestern Russia, the Balkans, the Middle East, central Asia, western China, and Africa, and between 1998 and 2013, CCHF was reported most frequently in Turkey, Russia, Iran, Pakistan, and Afghanistan.[26,27] The incidence of CCHF appears to be increasing, and possible causes include alterations in agricultural and domestic animal practices and climate changes.[28]

Hantaviruses

Hantaviruses cause two forms of severe acute illness: hemorrhagic fever with renal syndrome (HFRS), caused by hantaviruses of the Old World, and hantavirus cardiopulmonary syndrome (HCPS), also known as hantavirus pulmonary syndrome (HPS), caused by hantaviruses of the New World. These agents are fundamentally parasites of wild rodents and insectivores.[29] As such, hantaviruses are the exception to the general rule that bunyaviruses are arthropod-borne viruses. Although many rodent species worldwide have been shown to be infected, each of the presently recognized viral species has a single major rodent host species. This species becomes chronically infected despite an immune response that eliminates viremia, and its members excrete virus in urine and saliva for weeks or months.[30,31] Mechanisms of intraspecific transmission depend largely on horizontal transmission between sexually mature animals.[31] Hantaan virus (HTNV) is the cause of HFRS in Korea, China, and eastern Russia and is carried by the striped field mouse, *Apodemus agrarius*. *A. agrarius* is found in or near cultivars of humans; rodent breeding seasons and human agricultural practices result in fall and spring disease peaks.[32,33] Dobrava virus, associated with *Aedes flavicollis,* is the major cause of severe HFRS in the Balkans, and related viruses cause similarly severe disease in other areas of the former Soviet Union. Another hantavirus, Seoul virus (SEOV), is found worldwide in *Rattus norvegicus*. Although the virus is found wherever the reservoir sewer rat occurs, disease has rarely, if ever, been identified in the United States.

Bank voles, *Clethrionomys glareolus,* are the reservoir-vectors of Puumala virus, the cause of a milder form of HFRS termed *nephropathia epidemica* in Scandinavia, the western former Soviet Union, and Europe. These small rodents are found in forests and agricultural hedgerows, have highly fluctuating populations, and disperse into rural and suburban gardens and dwellings, particularly in the fall and winter of years when their populations reach peaks.[32,33]

Many native North and South American rodents (family Muridae, subfamily Sigmodontinae) host phylogenetically distinct hantaviruses associated with HPS.[34] HPS is a disease of the Americas and is probably more important in South America than in North America. The most important North American hantavirus is SNV. The reservoir of SNV is the deer mouse, *Peromyscus maniculatus,* a species that is widespread in the United States and readily enters homes and other structures. On the East Coast, the closely related New York virus causes chronic infection of the white-footed mouse, *Peromyscus leucopus*. Somewhat more distantly related viruses are Bayou and Black Creek Canal viruses found in the southern United States and Florida, respectively, and are associated with a degree of renal failure in their clinical picture. The most important South American virus is Andes virus, which is a common cause of disease in Argentina and Chile and is the only hantavirus that has caused person-to-person transmission.[34]

Transmission to Humans

California Encephalitis Virus Group

LACV transmission occurs through the bite of female mosquitoes that have viral infection of their salivary glands. Human infection occurs mainly during the summer and early fall in persons entering forested areas for recreation or those living near forests. Members of *A. triseriatus* (eastern tree-hole mosquito) range a considerable distance from forest across open terrain in search of a blood meal and breed effectively in some manufactured containers, such as abandoned tires, bringing the mosquito range closer to human habitation.[35] Jamestown Canyon virus has also been isolated from multiple mosquito species and various mammals (see later).

Rift Valley Fever

RVF in Africa has two main modes of transmission. It is recognized as a disease of farmers, veterinarians, and abattoir workers who have close contact with blood shed from sick domestic livestock or fresh carcasses containing a high concentration of virus.[18] Another major route of transmission to humans is from mosquito bites, particularly during epidemics. Infrequent years of heavy precipitation trigger the dormant transovarially infected eggs, and other secondary vectors widely disseminate virus.[18,22]

Crimean-Congo Hemorrhagic Fever

CCHFV infects humans principally by the bite of adult *Hyalomma* ticks. Milkers and shepherds are frequent victims. Asymptomatically viremic sheep and cattle have been implicated in transmission to abattoir workers, even outside known endemic areas,[36] and it is also hazardous to crush infected ticks. Highly infectious blood from patients also has caused several alarming nosocomial outbreaks with fatalities in medical personnel, particularly when the correct diagnosis of the index case was not suspected.[37–39] These have followed direct contact with blood and body fluids[40,41] and with aerosol generating procedures.[42]

Hantaviruses

Aerosols of virus-contaminated rodent urine or perhaps feces are thought to represent the principal vehicle for the transmission of hantaviruses; disease has also followed the bite of infected rodents because saliva contains virus, although this appears to be much less frequent.[43,44] Infections from *Apodemus* or *Clethrionomys* are acquired principally by persons visiting or working in forests and on farms. Depending on the circumstances, the incidence may be highest in summer or in fall and early winter. Disease is maximal in "high-rodent" years, when suburban residents may be exposed to disposing of infected rodents.[32–34,45]

Deer mice are numerous and readily enter human dwellings and outbuildings, particularly when mouse populations are high or in autumn when food and cover are scarce. Abundant rodent populations led to a large number of cases in the southwestern United States in the summer of 1993 and resulted in the first discovery of the virus. Most hantavirus epidemic years have been associated with increased rodent populations.[33,34]

CLINICAL MANIFESTATIONS

California Encephalitis Virus Group

Infection of humans by CE viruses is most commonly asymptomatic. After an incubation period of 3 to 7 days, however, individuals may experience mild febrile illness, encephalitis, or meningoencephalitis. Greater than 90% of acute central nervous system (CNS) disease caused by LACV occurs in children younger than 15 years; males are affected more often than females, and the mortality in acute CNS disease is about 1%.[1,10,46–48] LACV infection has caused encephalitis in an immunocompromised adult with a presentation resembling herpes simplex encephalitis.[47] Clinically and pathologically, CE is difficult to distinguish from other acute viral infections of the CNS. It can range in severity from mild aseptic meningitis to a severe disease mimicking herpes simplex encephalitis. Computed tomographic scans are abnormal in a minority of cases, magnetic resonance imaging is sometimes positive, and either can yield focal images.[10] The electroencephalogram is abnormal in two-thirds of patients, often with focal findings that include periodic lateralizing epileptiform discharges (PLEDs) that lead to a suspicion of herpes simplex encephalitis.[48] PLEDs often localize to the temporal lobe and are associated with more severe disease (e.g., convulsions, intubation, prolonged intensive care unit stay) as well as sequelae (e.g., epilepsy, cognitive and memory deficits).[49] Fever, headache, nausea, and vomiting are present in most patients. Lethargy, aphasia, incoordination, and focal motor abnormalities, even paralysis, may be present, but the outstanding serious finding is convulsions, which occur in about one-half of cases. The cerebrospinal fluid (CSF) generally shows a modest pleocytosis (<100 white blood cells [WBCs]/mm^3) that occasionally is largely granulocytic and exhibits a normal or slightly increased protein concentration. Peripheral leukocytosis in excess of 15,000 WBCs/mm^3 is not uncommon. Although most patients make uneventful recoveries, abnormal electroencephalographic findings 1 to 5 years later are present in 75%, emotional lability is persistent in 10%, and epilepsy is a chronic problem in 6% to 10% of all diagnosed cases. Frank neurologic deficit is uncommon but does occur.[46] Thus the residua of LAC encephalitis may be more serious than is generally appreciated. A possible congenital infection with LACV has been reported, without apparent abnormalities to the neonate. LACV infection during pregnancy has been associated with teratogenic effects in rabbits, gerbils, and sheep.[50–52]

Rift Valley Fever

RVFV infection in humans causes undifferentiated febrile disease in the great majority of instances. The incubation period lasts 2 to 6 days and is followed by fever and malaise, often accompanied by headache, photophobia, and back and joint pain.[53,54] Perhaps 10% of patients experience macular and perimacular retinitis, and vasculitis that may cause a permanent loss of vision. In as much as 1% of infections, fulminant disease with hemorrhage, jaundice, and hepatitis may develop at the end of a 3- to 6-day febrile episode, with high mortality.[55] Other infections (<1%) lead to severe, frequently fatal, encephalitis directly related to viral invasion of the CNS.

Crimean-Congo Hemorrhagic Fever

CCHF is generally a mild febrile illness but one that may progress to severe and fatal hemorrhagic disease.[56] CCHF has a distinct course of infection in humans with four different phases: incubation, prehemorrhagic, hemorrhagic, and convalescence period. After infection via tick bite, the incubation period is usually short, ranging on average between 1 and 5 days. The incubation period after contact with infected blood or tissues is usually 5 to 6 days, with a documented maximum of 13 days.[57] The first evidence of disease is usually a flushing of the face and the pharynx and a rash that progresses to petechiae, ecchymoses, hemorrhage of mucous membranes and conjunctiva, hematemesis, melena, epistaxis, hematuria, and hemoptysis.[57] Clinical manifestations in the prehemorrhagic period include fever, malaise, myalgia, dizziness, and, in some patients, diarrhea, nausea, and vomiting lasting for an average of about 3 days. The hemorrhagic phase usually begins on days 3 to 5 after the onset of illness and is usually short, lasting on average 2 to 3 days. Cerebral hemorrhage, gingival bleeding, and bleeding from the nose, vagina, uterus, or urinary tract may occur, as well as internal bleeding in abdominal muscles. Cerebral hemorrhage and massive liver necrosis indicate a poor prognosis. Hepatomegaly and splenomegaly may occur in up to 40% of the patients. Death generally occurs on days 5 to 14 of illness and is attributed to hemorrhages, hemorrhagic pneumonia, or cardiovascular disturbances. Lethal cases typically do not develop an antibody response. The mortality rate has been reported to average around 30% but may range from about 5% to greater than 80%.[58]

Hantaviruses

Hemorrhagic Fever With Renal Syndrome

The hallmarks of clinical infection by HTN, Dobrava, SEO, and Puumala viruses and other Eurasian hantaviruses are fever, thrombocytopenia, and acute renal insufficiency, pathologically typical of acute interstitial nephritis. The incubation period, typically 2 weeks, may vary from 5 to 42 days. In the severe form of HFRS exemplified by HTNV infection or Dobrava virus in Europe, patients who survive full-blown disease progress through febrile (toxic), hypotensive, oliguric, and polyuric clinical stages and may require weeks or months to recover from general asthenia.[59–61]

In the toxic phase patients complain of headache, abdominal and lower back pain, dizziness, and often blurred vision.[56–59] Conjunctival injection and petechiae occur over the upper trunk and soft palate. An erythematous flush that blanches on pressure is characteristically seen on the torso and face. Leukocyte levels are normal or more likely elevated, often exceeding 20,000/mm^3. The differential count shows a left shift, immature myeloid cells, and atypical lymphocytes as well, confirming the decreased thrombocyte count. At the end of the febrile period (4–7 days), many patients experience severe clinical shock. Those surviving then endure varied grades of renal insufficiency that can include anuria, oliguria, mucosal bleeding diathesis, electrolyte and acid-base abnormalities, hypertension, and pneumonitis complicated by pulmonary edema. After 3 to 10 days, polyuria begins with attendant stresses on the fluid and electrolyte balance. The fatality rate in severe HFRS caused by Hantaan or Dobrava viruses averages about 5%: one-third during the shock phases and two-thirds (cerebrovascular accidents and pulmonary edema) during the renal phases of illness.

Pathogenic hantaviruses enter endothelial cells via β3 integrins, which can result in disruption of cell-to-cell adhesion and in permeability alterations. This may result in an acute capillary leak syndrome, which accounts for varied clinical manifestations that are seen in hantavirus-associated diseases,[62,63] including hemodynamic changes. The renal lesions, predominantly in medullary tubules, may be related to both systemic and intrarenal hemodynamic factors and to the influence of immunopathologically released kinins and cytokines.[64] Bradykinin has been implicated in this regard,[65,66] and complement activation has been noted, involving both classical and alternative pathways.[67,68] Complement components C3 and C5 appear to be linked to observed disorders of coagulation and fibrinolysis cascades.[69]

The milder form of HFRS caused by Puumala virus, often referred to as *nephropathia epidemica*, is rarely hemorrhagic and is fatal in less than 1% of clinical cases. Abdominal pain and renal dysfunction may be manifestations. Up to 90% of Puumala virus infections are asymptomatic. Proteinuria, creatinine elevation, and leukocytosis are common but are much less severe than in HTNV infection.[70,71]

SEOV also causes a mild to moderately severe HFRS in Eurasia, with more prominent hepatic involvement than classic HFRS.[59,72]

Hantavirus Pulmonary Syndrome

HPS begins with a febrile prodrome, followed by a severe increase in pulmonary vascular permeability and shock.[64,73,74] If hypoxia is managed and shock is not fatal, the vascular leak reverses in a few days and recovery is virtually complete, although renal and other sequelae have

been suggested.[75] The first symptoms are fever of sudden onset and generalized myalgia. This prodrome resembles the initial phases of HFRS and may also be accompanied by abdominal pain and gastrointestinal (GI) disturbances.[73] About 4 to 5 days later (range, 1–10 days) the patient presents with respiratory symptoms, which usually consist of modest cough and dyspnea. Examination may be unrevealing, but in general fever, tachycardia, and tachypnea are present, perhaps with mild hypotension. Laboratory abnormalities commonly found at this time or developing within 1 to 2 days thereafter are an elevated hematocrit; leukocytosis, left shift, or both; abnormal (atypical) lymphocytes and immature myeloid cells on smear; mild thrombocytopenia; a prolonged activated partial thromboplastin time; and mildly elevated aspartate aminotransferase or lactate dehydrogenase (LDH) levels. Mild increases in serum creatinine levels and proteinuria occur in some cases,[34] but the severe renal lesions seen in HFRS are not a regular feature of this syndrome.[64,76] Respiratory involvement can progress from mild desaturation and interstitial pulmonary edema to florid pulmonary edema with respiratory failure in a matter of hours.[74,77] HPS should be suspected when an otherwise healthy adult develops unexplained pulmonary edema or is suspected of adult respiratory distress syndrome without one of the known causes of this syndrome being present; thrombocytopenia or a decreasing platelet count is a particularly useful finding early in the course.[74] Extracorporeal membrane oxygenation may improve survival but has never been tested in a controlled trial.[78]

The histopathologic findings of interstitial infiltrates of T lymphocytes and alveolar pulmonary edema without marked necrosis or polymorphonuclear leukocyte involvement, plus the rapid resolution of the lesion, suggest that the major abnormality may be the induction of a functional vascular permeability increase via an immunopathologic mechanism.[76,79]

DIAGNOSIS

A wide variety of laboratory tests have been developed to establish a diagnosis of infections with bunyaviruses. The applicability and availability of laboratory tests vary with the ecology and pathobiology of individual bunyaviruses. Serologic diagnosis is most frequently used for many bunyavirus infections, and most patients will have serum immunologic evidence of infection once they present clinically. The presence of virus-specific IgM may provide evidence for a recent infection but is generally less specific than a rise in IgG titers in acute and convalescent serum specimens.

Virus detection by conventional tissue culture techniques is difficult for most bunyaviruses. However, viral detection by highly sensitive and specific molecular techniques for viral detection have now been developed for many bunyaviruses.

Availability of and expertise with individual bunyavirus laboratory tests vary with geographic locale and frequency of infections encountered. Readers are urged to consult local public health authorities if questions arise.

Laboratory diagnosis for individual groups of bunyaviruses are described later.

California Encephalitis

The diagnosis of CE is by serologic means because virus is usually not present in blood or secretions by the time patients present with the clinical phase of CNS disease. The diagnosis can be rapidly and specifically achieved with ELISAs for antiviral IgM antibodies in blood and CSF, which are usually, but not always, positive at the time of admission.[10,80] A licensed indirect fluorescent antibody test is available for IgG and IgM antibodies to LACV and may be useful in the diagnosis.[11] An HI assay is also available and may be more sensitive but may require neutralization assays for confirmation.[81]

Viruses may also be detected by reverse-transcriptase polymerase chain reaction (RT-PCR) and multiplex nucleotide sequencing.[3] Virus isolation can be attempted by intracerebral inoculation of suckling mice or tissue culture in Vero cells.[3]

Riff Valley Fever and Congo-Crimea Hemorrhagic Fever Viruses

RVFV and CCHFV are readily recovered from the blood of acutely ill patients in cell cultures or suckling mice. Antigen-detection ELISA is useful in diagnosis, particularly in severe cases. The RT-PCR assay provides additional sensitivity with no loss of specificity. Antibodies detectable by a variety of methods generally appear within 5 to 14 days of onset and coincide with clinical improvement. ELISA detection of IgM antibodies is a reliable, definitive method.[18,36,37,82] Because of the aerosol hazard to laboratory personnel, acute samples must be handled with care, and attempts to isolate these two agents should be restricted to facilities with maximal containment.

Hantaviruses

Virtually all hantavirus patients have both IgM and IgG ELISA antibodies present when admitted to the hospital.[60] Hantaviruses can be recovered only with difficulty in cell culture or animal hosts,[31] but the agent can be detected in blood or tissues by RT-PCR or in tissues by immunohistochemical staining.[76,79,83]

THERAPY

Therapy with the antiviral drug Ribavirin, a guanosine analogue, has been shown to be effective in the treatment of HFRS in a double-blind placebo-controlled study in China using the intravenous dosing regimen established for Lassa fever (see Chapter 167). Intensive monitoring and frequent dialysis, even without ribavirin, result in very low mortality. An open-label trial of ribavirin failed to show any efficacy in HPS patients, perhaps because death typically occurs within 24 to 48 hours of hospitalization.[75]

Studies in vitro and in laboratory animals suggest that ribavirin also might be effective in the treatment of severe RVF and CCHF, and observational clinical experience with the drug in CCHF supports its use.[84,85] However, a recent Cochrane Database review states that there is "insufficient evidence to show whether ribavirin is effective in treating CCHF" and that appropriately designed clinical trials are necessary.[86,87] Ribavirin also has activity in vitro against LACV, and treatment of one unusual case of LACV infection diagnosed with brain biopsy has been reported.[88]

The possible effectiveness of treatment with immune plasma was examined in HPS in 29 confirmed cases in Chile (2008–12). The case fatality rate was 4 per 29 (14%) in cases treated with convalescent plasma, compared with 18 per 66 (27%) in HPS cases who were not treated with plasma at the same study sites ($P = .15$).[89] Further studies are required to investigate this observation.

Effective supportive care is important in all of the severe bunyavirus diseases. Careful management of coma, cerebral edema, and seizures is critical in CE patients; there is danger in too vigorous use of phenobarbital in children with status epilepticus.[10,48,88] Early management of hantavirus patients should avoid excessive administration of fluids in these febrile, hemoconcentrated, hypotensive patients. Vascular leak leads to extravasation into retroperitoneal tissues (HFRS) or lung (HPS); cardiotonic drugs should be used early because of the hemodynamic profile of decreased cardiac output and increased systemic vascular resistance.[64] Patients with severe HFRS may require hemodialysis or peritoneal dialysis during the oliguric phase, and plasma protein or whole blood, or both, may be useful in treating hemorrhage or shock, or both, in this and other hemorrhagic fevers. Heparin is not recommended for the treatment of presumptive or incipient disseminated intravascular coagulation in HFRS. Patients with mild HFRS due to Puumala virus rarely require dialysis.

PREVENTION

Prevention of bunyavirus diseases currently is accomplished primarily by personal means (e.g., avoidance of rodent contact, use of mosquito and tick repellents), and perhaps, in the case of LACV, by the elimination of manufactured containers leading to mosquito breeding, together with aerial spraying of slow-release insecticides over forested areas of known high *A. triseriatus* reproduction.[90]

A variety of vaccines against a number of bunyaviruses have been developed and studied, but none have received widespread usage. Immunization of livestock against RVFV is carried out with formalin inactivated and live-attenuated vaccines in parts of Africa.[91,92] An inactivated suckling mouse–derived vaccine against CCHFV has been developed in Eastern Europe,[93] and CCHFV glycoprotein vaccines are

also under study.[94] Several inactivated virus vaccines have been developed against SEOV and HTNV in Asia,[95] and DNA gene–based vaccines against hantaviruses are also under study.[3]

OTHER BUNYAVIRALES OF CONCERN

Jamestown Canyon Virus

Jamestown Canyon virus (JCV) is a member of the CE group of bunyaviruses that have been a likely unrecognized cause of infections and CNS disease in North America.[96,97] JCV was first identified in Jamestown Canyon, Colorado in 1961[98] and has been recovered from various mosquito species: *Aedes, Coquillettidia, Culex,* and *Culiseta*. Burgeoning populations of whitetail deer have been suspected as a vertebrate amplifier. Serologic surveys in humans and animals indicate that JCV is widespread through North America. A recent analysis of 31 laboratory-confirmed infections of JCV indicated that most infections occurred in spring to early fall and involved all age groups.[96] Clinically recognized infections present as an undifferentiated febrile illness, meningitis, or meningoencephalitis. Approximately half of identified infections required hospitalization, and deaths have not been reported to date.[96] The rate of asymptomatic infections with JCV is not known.[97]

Partial cross-reactions exist between LACV and JCV IgM responses and may require plaque neutralization or RT-PCR tests to differentiate between infections with these.

Oropouche Virus

Oropouche virus has been associated with epidemics and sporadic cases of febrile illness in tropical areas of Brazil, Panama, and Peru.[99–101] It is transmitted by biting midges *(Culicoides paraensis).* In rural areas transmission may also involve nonhuman primates, sloths, birds, and mosquitoes. In urban areas Oropouche virus can be transmitted between viremic individuals and biting midges.[101–104] The risk of transmission appears to be highest during the rainy season, during which breeding of midges is increased.[105] *C. paraensis* has been identified in states east of the Mississippi River, but the likelihood of establishment of ecologic factors for virus transmission in the United States is low.

The incubation period of disease in human ranges from 3 to 12 days.[99,100,106] Infection results in an abrupt onset of fever, chills, headache, myalgia, and often vomiting and arthralgia. Aseptic meningitis has been reported in some cases. Symptoms last for 4 to 5 days and are usually self-limiting. However, prolonged asthenia and arthralgia can occur, and recurrences 10 days after recovery have been observed.[101]

Toscana Virus

The classic sand fly fevers (Sicilian and Naples viruses) are acute febrile illnesses with headache and myalgias and were common in the broad European and Asian range of their vector, *Phlebotomus papatasi,* until dichlorophenyltrichloroethane (DDT) campaigns against malaria virtually eliminated this sand fly in much of Europe. Another sand fly, *Phlebotomus perniciosus,* spreads the related but distinct Toscana virus, which appears to be a common cause of infections in Tuscany and areas of Southern Europe.[107–110] High seroprevalence rates to Toscana virus in endemic areas suggest that most infections are asymptomatic or are of minimal symptomatology. However, Toscana virus can cause febrile disease, aseptic meningitis, or mild encephalitis. Overall prognosis is good, although severe cases have been reported. CNS infections are common in the circum-Mediterranean distribution of the vector in Europe, from Cyprus to Portugal and Spain, and are often causes of disease in returning travelers.[107,111,112] Diagnosis can usually be made serologically by detection of virus-specific IgM by ELISA and immunofluorescence assays, or by virus neutralizing antibody rises.[113]

Ngari Virus

During the 1997–98 RVF outbreak in Kenya, two viruses belonging to the *Orthobunyavirus* genus were isolated from putative hemorrhagic fever cases, and RT-PCR was positive in 12 sera. The virus was found to have the S and L RNA segments of Bunyamwera virus, but the M segment was derived from another bunyavirus, subsequently identified as Ngari virus.[114] Subsequent complete genomic sequencing of Ngari virus indicated that Ngari virus was a naturally occurring reassortant between two orthobunyaviruses, Bunyamwera and Batai viruses.[115,116] S and L segments were derived from Bunyamwera virus, and M segments were derived from Batai virus. Thus this virus should be considered in other viral hemorrhagic fever epidemics and is a cautionary example of the emergence of novel pathogens through viral reassortment.[117]

Severe Fever With Thrombocytopenia Syndrome Virus

A new member of the *Phlebovirus* genus was isolated in China (Hubei and Henan provinces) from patients who presented with fever, thrombocytopenia, leukocytopenia, and multiorgan dysfunction and named severe fever with thrombocytopenia syndrome virus (SFTSV).[118] The clinical symptoms were initially considered to resemble those of human granulocytic anaplasmosis.[119] The majority of affected patients were farmers living in wooded and hilly areas, and they were working in the fields before onset of clinical signs of disease. SFTSV RNA has been detected in ticks of the Ixodidae *(Haemaphysalis longicornis, Ixodes nipponensis, Amblyomma testudinarium)* that were collected from domestic animals where patients lived. Initially, no epidemiologic evidence of human-to-human transmission of SFTSV was detected.[118] However, subsequent publications indicated that the virus can be transmitted from person-to-person through close personal contact with the index patient without known exposure to suspected animals or vectors.[120–122] Nosocomial transmission in hospital settings has also been reported.[123]

SFTSV appears to be most closely related to the Bhanja serogroup among members of the *Phlebovirus* genus.[124] SFTSV has been detected in central and eastern China, rural areas of South Korea, and Western Japan.[125,126]

SFTS has an incubation period of 7 to 14 days and begins with a febrile illness, malaise, headache, GI disturbances, myalgias, and arthralgias. During the second week of illness, multiorgan dysfunction can develop, with renal dysfunction, myocarditis, and CNS involvement.[127–130] Bleeding manifestations, including disseminated intravascular coagulation and mucosal hemorrhage, can ensue. Disease manifestations may begin to resolve over 1 to 2 weeks, but cases can continue to worsen, and overall case fatality rates have been estimated to be 6% to 21%.[129,131,132] Laboratory abnormalities include leukopenia, thrombocytopenia, elevated liver function tests, and elevated LDH and creatine phosphokinase levels.

The pathogenesis of SFTS appears to be related to high viral loads, which results in a systemic inflammatory response. Marked upregulation of multiple cytokines has been correlated with disease severity.[133–135]

Laboratory diagnosis during the first week of illness can be made by detection of SFTSV in blood by RT-PCR[134] or by loop-mediated isothermal amplification.[135]

Therapy consists of general medical support and measures to treat bleeding diathesis. Ribavirin has in vitro activity against SFTSV and has been used to treat patients with SFTS in China, without apparent effectiveness.[136] Favipiravir has been shown to be effective in vitro and in a mouse model against SFTSV (also see Chapter 45).

Heartland Virus

Heartland virus is a newly recognized pathogenic bunyavirus in the *Phlebovirus* genus, first reported from two cases in Missouri in 2012.[137] As of 2017 30 cases have been reported to the Centers for Disease Control and Prevention, all from the midwestern and southern United States.[137,139] Transmission appears likely to be tick-borne, and Heartland virus has been detected by PCR and culture in nymphs of the Lone Star tick *(Amblyomma americanum),* which can be found in the United States from Texas to Maine.[140] Neutralizing antibodies to Heartland virus have been found in a wide variety of vertebrate life, including opossums, dogs, raccoons, deer, moose, horses, and coyotes,[141,142] but the major vertebrate host has not been identified. Human-to-human transmission has not been reported, although such has been observed in infection by a related *Phlebovirus* (SFTSV) (see earlier).

Clinical manifestations of Heartland virus–associated disease consist of fever, headache, GI disturbances, dry cough, confusion, myalgias, and arthralgias.[138] Fatigue and memory loss lasting weeks to months has been described.[138] Widespread dissemination[143] and fatal cases have been reported.[144,145] Laboratory findings include leukopenia, thrombocytopenia, and elevated liver enzymes.[143,146] A fatal case of Heartland virus infections

with hemophagocytic lymphohistiocytosis in an immunocompromised patient has been described.[147]

Laboratory diagnosis of Heartland virus infection can be made by virus detection by RT-PCR or tissue culture, or serologically by ELISA or plaque reduction neutralization tests.[148]

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Key References

The complete reference list is available online at Expert Consult.

1. Adams MJ, Lefkowitz EJ, King AMQ, et al. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses. *Arch Virol*. 2017;162:2505–2538.
2. International Committee on Taxonomy of Viruses (ICTV). Taxonomy. https://talk.ictvonline.org/taxonomy/. Accessed November 19, 2018.
3. Elliot RM, Schmaljohn CS. Bunyaviridae. In: Knipe DM, Howley PM, eds. *Fields Virology*. Philadelphia: Lippincott Williams & Wilkins; 2013:1244–1282.
4. Wichgers Schreur PJ, Kormelink R, Kortekaas J. Genome packaging of the Bunyavirales. *Curr Opin Virol*. 2018;33: 151–155.
5. Guardado-Calvo P, Rey FA. The envelope proteins of the Bunyavirales. *Adv Virus Res*. 2017;98:83–118.
6. Goldsmith CS, Elliott LH, Peters CJ, et al. Ultrastructural characteristics of Sin Nombre virus, causative agent of hantavirus pulmonary syndrome. *Arch Virol*. 1995;140:2107.
7. Thompson WH. Vector-virus relationship. In: Calisher CH, Thompson WH, eds. *California Serogroup Viruses, Proceedings of an International Symposium*. New York: Alan R Liss; 1983:57.
12. Centers for Disease Control and Prevention. Possible congenital infection with La Crosse encephalitis virus—West Virginia, 2006-2007. *MMWR Morb Mortal Wkly Rep*. 2009;58:4–7.
13. Cully JF, Streit TG, Geard PB. Transmission of La Crosse virus by four strains of *Aedes albopictus* and from the eastern chipmunk *(Tamias striatus)*. *J Am Mosq Control Assoc*. 1992;8:237.
14. Gerhardt RR, Gottfried KL, Apperson CS, et al. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerg Infect Dis*. 2001;7:807–811.
15. Eldridge BF, Glaser C, Pedrin RE, et al. The first reported case of California encephalitis in more than 50 years. *Emerg Infect Dis*. 2001;7:451–452.
16. Huang CW, Campbell L, Grady I, et al. Diagnosis of Jamestown Canyon encephalitis by polymerase chain reaction. *Clin Infect Dis*. 1999;28:1294–1297.
17. Lithicum KJ, Davies FG, Kairo A, et al. Rift Valley fever virus (family Bunyaviridae, genus *Phlebovirus*). Isolation from Diptera collected during an interepizootic period in Kenya. *J Hyg (Lond)*. 1985;95:197.
18. Swanepoel R, Coetzer JAW. Rift Valley fever. In: Coetzer JAW, Tustin RC, eds. *Infectious Diseases of Livestock*. 2nd ed. Oxford: Oxford University Press; 2004:1037–1070.
19. Woods CW, Karpati AM, Grein T, et al. An outbreak of Rift Valley fever in Northeastern Kenya, 1997-98. *Emerg Infect Dis*. 2002;8:138–144.
22. Peters CJ. Emergence of Rift Valley fever. In: Saluzzo JF, Dodet B, eds. *Factors in the Emergence of Arbovirus Diseases*. Paris: Elsevier; 1997:253.
23. van Eeden PJ, Joubert JR, van de Wal BW, et al. A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part I. Clinical features. *S Afr Med J*. 1985;68:711–717.
24. Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2004;64:145–160.
25. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J Med Entomol*. 1979;15:307–417.
26. Leblebicioglu H, Ozaras R, Sunbul M. Crimean-Congo hemorrhagic fever: a neglected infectious disease with potential nosocomial infection threat. *Am J Infect Control*. 2017;45:815–816.
27. Ince Y, Yasa C, Metin M, et al. Crimean-Congo hemorrhagic fever infections reported by ProMED. *Int J Infect Dis*. 2014;26:44–46.
28. Leblebicioglu H, Ozaras R, Erciyas-Yavuz K. Emergence of Crimean-Congo hemorrhagic fever. *Trans R Soc Trop Med Hyg*. 2015;109:676–678.
29. Peters CJ, Mills JN, Spiropoulou C, et al. Hantavirus infections. In: Guerrant RL, Walker DH, Weller PF, eds. *Tropical Infectious Diseases—Principles, Pathogens and Practice*. 2nd ed. Philadelphia: Churchill Livingstone; 2006:762–780.
30. Lee HW, Lee PW, Baek LJ, et al. Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent *Apodemus agrarius*. *Am J Trop Med Hyg*. 1981;30:1106.
31. Hutchinson KL, Rollin PE, Peters CJ. Pathogenesis of a North American hantavirus, Black Creek Canal virus, in experimentally infected *Sigmodon hispidus*. *Am J Trop Med Hyg*. 1998;59:58.
34. Peters CJ. Hantavirus pulmonary syndrome in the Americas. In: Scheld WM, Craig WA, Hughes JM, eds. *Emerging Infections II*. Washington, DC: American Society for Microbiology Press; 1998:7–64.
35. Mather TN, DeFoliart GR. Dispersion of gravid *Aedes triseriatus* (Diptera: Culicidae) from woodlands into open terrain. *J Med Entomol*. 1984;21:384.
36. Rodriguez LL, Maupin GO, Ksiazek TG, et al. Molecular investigation of a multisource outbreak of Crimean-Congo hemorrhagic fever in the United Arab Emirates. *Am J Trop Med Hyg*. 1997;57:512.
37. Burney MI, Ghafoor A, Saleen M, et al. Nosocomial outbreak of viral hemorrhagic fever—Congo virus in Pakistan, January 1976. *Am J Trop Med Hyg*. 1980;29:941.
40. Sunbul M, Leblebicioglu H, Fletcher TE, et al. Crimean-Congo haemorrhagic fever and secondary bacteraemia in Turkey. *J Infect*. 2015;71:597–599.
41. Leblebicioglu H, Sunbul M, Guner R, et al. Healthcare-associated Crimean-Congo haemorrhagic fever in Turkey, 2002-2014: a multicentre retrospective cross-sectional study. *Clin Microbiol Infect*. 2016;22:387, e1–e4.
42. Pshenichnaya NY, Nenadskaya SA. Probable Crimean-Congo hemorrhagic fever virus transmission occurred after aerosol-generating medical procedures in Russia: nosocomial cluster. *Int J Infect Dis*. 2015;33: 120–122.
43. Kawamata J, Yamanouchi T, Dohmae K, et al. Control of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) in Japan. *Lab Anim Sci*. 1987;37:431.
44. Hjelle B, Glass GE. Outbreak of hantavirus infection in the Four Corners region of the United States in the wake of the 1997-1998 El Niño-southern oscillation. *J Infect Dis*. 2000y;181:1569–1573.
46. McJunkin JE, de los Reyes EC, Irazuzta JE, et al. La Crosse encephalitis in children. *N Engl J Med*. 2001;344:801–807.
47. Wurtz R, Paleologos N. La Crosse encephalitis presenting like herpes simplex encephalitis in an immunocompromised adult. *Clin Infect Dis*. 2000;31:1113–1114.
48. Deering WM. Neurological aspects and treatment of La Crosse encephalitis. In: Calisher CH, Thompson WH, eds. *California Serogroup Viruses. Proceedings of an International Symposium*. New York: Alan R Liss; 1983:187.
49. de los Reyes EC, McJunkin JE, Glauser TA, et al. Periodic lateralized epileptiform discharges in La Crosse encephalitis, a worrisome subgroup: clinical presentation, electroencephalogram (EEG) patterns, and long-term neurologic outcome. *J Child Neurol*. 2008;23:167–172.
50. Tsai TF. Congenital arboviral infections: something new, something old. *Pediatrics*. 2006;117:936–939.
53. Bartelloni PJ, Tesh RB. Clinical and serologic responses of volunteers infected with phlebotomus fever virus (Sicilian type). *Am J Trop Med Hyg*. 1976;25:456–462.
54. Sabin AB. Experimental studies on *Phlebotomus* (pappataci, sandfly) fever during World War II. *Arch Gesamte Virusforsch*. 1951;4:367–410.
55. Al Hazmi M, Ayoola EA, Abdurahman M, et al. Epidemic Rift Valley fever in Saudi Arabia: a clinical study of severe illness in humans. *Clin Infect Dis*. 2003;36:245–252.
57. Ergonul O. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis*. 2006;6:203–214.
58. Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2004;64:145–160.
59. Lee JS, Cho BY, Lee MC, et al. Clinical features of serologically proven Korean hemorrhagic fever patients. *Seoul J Med*. 1980;21:163.
62. Gavrilovskaya IN, Brown EJ, Ginsberg MH, et al. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. *J Virol*. 1999;73:3951–3959.
63. Gavrilovskaya IN, Peresleni T, Geimonen E, et al. Pathogenic hantaviruses selectively inhibit beta3 integrin directed endothelial cell migration. *Arch Virol*. 2002;147: 1913–1931.
64. Peters CJ, Simpson G, Levy H. Spectrum of hantavirus infection: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. *Annu Rev Med*. 1999;50:531–545.
65. Taylor SL, Wahl-Jensen V, Copeland AM, et al. Endothelial cell permeability during hantavirus infection involves factor XII-dependent increased activation of the kallikrein-kinin system. *PLoS Pathog*. 2013;9:e1003470.
66. Antonen J, Leppänen I, Tenhunen J, et al. A severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant. *Scand J Infect Dis*. 2013;45:494.
67. Paakkala A, Mustonen J, Viander M, et al. Complement activation in nephropathia epidemica caused by Puumala hantavirus. *Clin Nephrol*. 2000;53:424.
68. Sane J, Laine O, Mäkelä S, et al. Complement activation in Puumala hantavirus infection correlates with disease severity. *Ann Med*. 2012;44:468.
69. Amara U, Flierl MA, Rittirsch D, et al. Molecular intercommunication between the complement and coagulation systems. *J Immunol*. 2010;185:5628.
70. Mäkelä S, Ala-Houhala I, Mustonen J, et al. Renal function and blood pressure five years after Puumala virus-induced nephropathy. *Kidney Int*. 2000;58:1711.
71. Miettinen MH, Mäkelä SM, Ala-Houhala IO, et al. Ten-year prognosis of Puumala hantavirus-induced acute interstitial nephritis. *Kidney Int*. 2006;69:2043.
72. Kim YS, Ahn C, Han JS, et al. Hemorrhagic fever with renal syndrome caused by the Seoul virus. *Nephron*. 1995;71:419–427.
73. Duchin JS, Koster F, Peters CJ, et al. Hantavirus pulmonary syndrome: clinical description of disease caused by a newly recognized hemorrhagic fever virus in the southwestern United States. *N Engl J Med*. 1994;330:949.
74. Moolenaar RL, Dalton C, Lipman HB, et al. Clinical features that differentiate hantavirus pulmonary syndrome from three other acute respiratory illnesses. *Clin Infect Dis*. 1995;21:643.
76. Peters CJ, Khan AS. Hantavirus pulmonary syndrome: the new American hemorrhagic fever. *Clin Infect Dis*. 2002;34:1224–1231.
79. Zaki SR, Greer PW, Coffield LM, et al. Hantavirus pulmonary syndrome: pathogenesis of an emerging infectious disease. *Am J Pathol*. 1995;146:552.
80. Calisher CH, Pretzman CI, Muth DJ, et al. Serodiagnosis of La Crosse virus infections in humans by detection of immunoglobulin M class antibodies. *J Clin Microbiol*. 1986;12:667.
81. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science*. 1998;281:1305–1308.
82. Swanepoel R. Crimean-Congo haemorrhagic fever. In: Palmer SR, Soulsby EJL, Simpson DIH, eds. *Zoonoses*. Oxford: Oxford University Press; 1998:461–470.
83. Nichol ST, Spiropoulou CF, Morzunov S, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science*. 1993;262: 914–917.
84. Peters CJ, Reynolds JA, Slone TW, et al. Prophylaxis of Rift Valley fever with antiviral drugs, immune serum, an interferon inducer, and a macrophage activator. *Antiviral Res*. 1986;6:285.
85. Mardani M, Jahromi MK, Naieni KH, et al. The efficacy of oral ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Iran. *Clin Infect Dis*. 2003;36:1613–1618.
86. Johnson S, Henschke N, Maayan N, et al. Ribavirin for treating Crimean Congo haemorrhagic fever. *Cochrane Database Syst Rev*. 2018;(6):CD012713.
87. Ceylan B, Turhan V. The efficacy of ribavirin in Crimean-Congo hemorrhagic fever–randomized trials are urgently needed. *Int J Infect Dis*. 2014;29:297.
88. McJunkin JE, Khan R, de los Reyes EC, et al. Treatment of severe La Crosse encephalitis with intravenous ribavirin following diagnosis by brain biopsy. *Pediatrics*. 1997;99:261.
89. Vial PA, Valdivieso F, Calvo M, et al. A non-randomized multicentre trial of human immune plasma for treatment of hantavirus cardiopulmonary syndrome caused by Andes virus. *Antivir Ther*. 2015;20:377–386.
90. Francy DB. Mosquito control for prevention of California (La Crosse) encephalitis. In: Calisher CH, Thompson WH, eds. *California Serogroup Viruses. Proceedings of an*

International Symposium. New York: Alan R Liss; 1983:365.
91. El-Karamany RM, Imam IZE, Farid AH, et al. Production of inactivated RVF vaccine. *J Egypt Pub Health Assoc*. 1981;56:495–525.
92. Coackley W, Pini A, Gosden D. The immunity induced in cattle and sheep by inoculation of neurotropic or pantropic Rift Valley fever viruses. *Res Vet Sci*. 1967;8:406–414.
93. Keshtkar-Jahromi M, Kuhn JH, Christova I, et al. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. *Antiviral Res*. 2011;90:85–92.
94. Buttigieg KR, Dowall SD, Findlay-Wilson S, et al. A novel vaccine against Crimean-Congo haemorrhagic fever protects 100% of animals against lethal challenge in a mouse model. *PLoS ONE*. 2014;9:e91516.
95. Hooper JW, Li D. Vaccines against hantaviruses. *Curr Top Microbiol Immunol*. 2001;256:171–191.
96. Pastula DM, Hoang Johnson DK, White JL, et al. Jamestown Canyon virus disease in the United States-2000-2013. *Am J Trop Med Hyg*. 2015;93:384–389.
97. Pastula DM, Smith DE, Beckham JD, et al. Four emerging arboviral diseases in North America: Jamestown Canyon, Powassan, chikungunya, and Zika virus diseases. *J Neurovirol*. 2016;22:257–260.
98. Centers for Disease Control and Prevention. CDC Arboviral Catalog. 2018. https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=206&SID=2. Accessed November 26, 2018.
99. Watts DM, Phillips I, Callahan JD, et al. Oropouche virus transmission in the Amazon River basin of Peru. *Am J Trop Med Hyg*. 1997;56:148.
101. Pinheiro FP, Travassos da Rosa AP, Travassos da Rosa JF, et al. Oropouche virus. I. A review of clinical, epidemiological, and ecological findings. *Am J Trop Med Hyg*. 1981;30:149.
102. Tesh RB. The emerging epidemiology of Venezuelan hemorrhagic fever and Oropouche fever in tropical South America. *Ann N Y Acad Sci*. 1994;740:129.
103. Mourãão MP, Bastos MS, Gimaqu JB, et al. Oropouche fever outbreak, Manaus, Brazil, 2007-2008. *Emerg Infect Dis*. 2009;15:2063.
104. Travassos da Rosa JF, de Souza WM, Pinheiro FP, et al. Oropouche virus: clinical, epidemiological, and molecular aspects of a neglected *Orthobunyavirus*. *Am J Trop Med Hyg*. 2017;96:1019.
105. Mourão MP, Bastos Mde S, Figueiredo RM, et al. Arboviral diseases in the Western Brazilian Amazon: a perspective and analysis from a tertiary health & research center in Manaus, State of Amazonas. *Rev Soc Bras Med Trop*. 2015;48(suppl 1):20.
106. World Health Organization. Oropouche Virus Disease—Peru. 2016. http://www.who.int/csr/don/03-june-2016-oropouche-peru/en/. Accessed August 16, 2016.
109. Charrel RN, Gallian P, Navarro-Mari JM, et al. Emergence of Toscana virus in Europe. *Emerg Infect Dis*. 2005;11:1657.
110. Di Nicuolo G, Pagliano P, Battisti S, et al. Toscana virus central nervous system infections in southern Italy. *J Clin Microbiol*. 2005;43:6186.
113. Pierro A, Ficarelli S, Ayhan N, et al. Characterization of antibody response in neuroinvasive infection caused by Toscana virus. *Clin Microbiol Infect*. 2017;23:868–873.
114. Gerrard SR, Li L, Barrett AD, et al. Ngari virus is a Bunyamwera virus reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa. *J Virol*. 2004;78:8922–8926.
115. Groseth A, Weisend C, Ebihara H. Complete genome sequencing of mosquito and human isolates of Ngari virus. *J Virol*. 2012;86:13846–13847.
116. Odhiambo C, Venter M, Lwande O, et al. Phylogenetic analysis of Bunyamwera and Ngari viruses (family Bunyaviridae, genus *Orthobunyavirus*) isolated in Kenya. *Epidemiol Infect*. 2016;144:389–395.
117. Bowen MD, Trappier SG, Sanchez AJ, et al. A reassortant bunyavirus isolated from acute hemorrhagic fever cases in Kenya and Somalia. *Virology*. 2001;291:185–190.
118. Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. *N Engl J Med*. 2011;364:1523–1532.
119. Zhang L, Liu W, Ni D, et al. Nosocomial transmission of human granulocytic anaplasmosis in China. *JAMA*. 2008;300:2263–2270.
120. Bao CJ, Guo XL, Qi X, et al. A family cluster of infections by a newly recognized bunyavirus in eastern China, 2007: further evidence of person-to-person transmission. *Clin Infect Dis*. 2011;53:1208–1214.
121. Gai ZT, Zhang Y, Liang MF, et al. Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. *J Infect Dis*. 2012;206:1095–1102.
122. Liu Y, Li Q, Hu W, et al. Person-to-person transmission of severe fever with thrombocytopenia syndrome virus. *Vector Borne Zoonotic Dis*. 2012;12:156–160.
123. Kim WY, Choi W, Park SW, et al. Nosocomial transmission of severe fever with thrombocytopenia syndrome in Korea. *Clin Infect Dis*. 2015;60:1681–1683.
124. Odhiambo C, Venter M, Lwande O, et al. Phylogenetic analysis of Bunyamwera and Ngari viruses (family Bunyaviridae, genus *Orthobunyavirus*) isolated in Kenya. *Epidemiol Infect*. 2016;144:389–395.
125. Liu Q, He B, Huang SY, et al. Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis. *Lancet Infect Dis*. 2014;14:763.
126. Takahashi T, Maeda K, Suzuki T, et al. The first identification and retrospective study of severe fever with thrombocytopenia syndrome in Japan. *J Infect Dis*. 2014;209:816.
127. Zhang YZ, He YW, Dai YA, et al. Hemorrhagic fever caused by a novel Bunyavirus in China: pathogenesis and correlates of fatal outcome. *Clin Infect Dis*. 2012;54:527.
128. Gai ZT, Zhang Y, Liang MF, et al. Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. *J Infect Dis*. 2012;206:1095.
129. Kato H, Yamagishi T, Shimada T, et al. Epidemiological and clinical features of severe fever with thrombocytopenia syndrome in Japan, 2013-2014. *PLoS ONE*. 2016;11:e0165207.
130. Guo CT, Lu QB, Ding SJ, et al. Epidemiological and clinical characteristics of severe fever with thrombocytopenia syndrome (SFTS) in China: an integrated data analysis. *Epidemiol Infect*. 2016;144:1345.
131. Sun J, Lu L, Wu H, et al. The changing epidemiological characteristics of severe fever with thrombocytopenia syndrome in China, 2011-2016. *Sci Rep*. 2017;7:9236.
132. Choi SJ, Park SW, Bae IG, et al. Severe fever with thrombocytopenia syndrome in South Korea, 2013-2015. *PLoS Negl Trop Dis*. 2016;10:e0005264.
133. Deng B, Zhang S, Geng Y, et al. Cytokine and chemokine levels in patients with severe fever with thrombocytopenia syndrome virus. *PLoS ONE*. 2012;7:e41365.
134. Sun Y, Jin C, Zhan F, et al. Host cytokine storm is associated with disease severity of severe fever with thrombocytopenia syndrome. *J Infect Dis*. 2012;206:1085.
135. Hu LF, Wu T, Wang B, et al. The regulation of seventeen inflammatory mediators are associated with patient outcomes in severe fever with thrombocytopenia syndrome. *Sci Rep*. 2018;8:159.
136. Sun Y, Liang M, Qu J, et al. Early diagnosis of novel SFTS bunyavirus infection by quantitative real-time RT-PCR assay. *J Clin Virol*. 2012;53:48.
137. McMullan LK, Folk SM, Kelly AJ, et al. A new phlebovirus associated with severe febrile illness in Missouri. *N Engl J Med*. 2012;367:834–841.
139. Centers for Disease Control and Prevention. Heartland Virus Disease. https://www.cdc.gov/ticks/tickbornediseases/heartland-virus.html. Accessed November 29, 2018.
140. Savage HM, Godsey MS Jr, Lambert A, et al. First detection of Heartland virus (Bunyaviridae: *Phlebovirus*) from field collected arthropods. *Am J Trop Med Hyg*. 2013;89:445.
141. Riemersma KK, Komar N. Heartland virus neutralizing antibodies in vertebrate wildlife, United States, 2009-2014. *Emerg Infect Dis*. 2015;21:1830.
142. Bosco-Lauth AM, Panella NA, Root JJ, et al. Serological investigation of Heartland virus (Bunyaviridae: *Phlebovirus*) exposure in wild and domestic animals adjacent to human case sites in Missouri 2012-2013. *Am J Trop Med Hyg*. 2015;92:1163.
143. Fill MA, Compton ML, McDonald EC, et al. Novel clinical and pathologic findings in a Heartland virus-associated death. *Clin Infect Dis*. 2017;64:510.
144. Muehlenbachs A, Fata CR, Lambert AJ, et al. Heartland virus-associated death in Tennessee. *Clin Infect Dis*. 2014;59:845.
145. Oklahoma State Department of Health. Oklahoma State Health Department Confirms First Case and Death of Heartland Virus, May 24, 2014. https://www.ok.gov/health/Organization/Office_of_Communications/News_Releases/2014_News_Releases/Oklahoma_State_Health_Department_Confirms_First_Case_and_Death_of_Heartland_Virus.html. Accessed November 29, 2018.
146. Pastula DM, Turabelidze G, Yates KF, et al. Notes from the field: Heartland virus disease - United States, 2012-2013. *MMWR Morb Mortal Wkly Rep*. 2014;63:270.
147. Carlson AL, Pastula DM, Lambert AJ, et al. Heartland virus and hemophagocytic lymphohistiocytosis in immunocompromised patient, Missouri, USA. *Emerg Infect Dis*. 2018;24:893–897.
148. Centers for Disease Control and Prevention. Clinical and Lab Evaluations, Heartland Virus. https://www.cdc.gov/heartland-virus/healthcare-providers/clinical-lab-evals.html. Accessed November 29, 2018.

References

1. Adams MJ, Lefkowitz EJ, King AMQ, et al. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses. *Arch Virol*. 2017;162:2505–2538.
2. International Committee on Taxonomy of Viruses (ICTV). Taxonomy. https://talk.ictvonline.org/taxonomy/. Accessed November 19, 2018.
3. Elliot RM, Schmaljohn CS. Bunyaviridae. In: Knipe DM, Howley PM, eds. *Fields Virology*. Philadelphia: Lippincott Williams & Wilkins; 2013:1244–1282.
4. Wichgers Schreur PJ, Kormelink R, Kortekaas J. Genome packaging of the Bunyavirales. *Curr Opin Virol*. 2018;33: 151–155.
5. Guardado-Calvo P, Rey FA. The envelope proteins of the Bunyavirales. *Adv Virus Res*. 2017;98:83–118.
6. Goldsmith CS, Elliott LH, Peters CJ, et al. Ultrastructural characteristics of Sin Nombre virus, causative agent of hantavirus pulmonary syndrome. *Arch Virol*. 1995;140: 2107.
7. Thompson WH. Vector-virus relationship. In: Calisher CH, Thompson WH, eds. *California Serogroup Viruses, Proceedings of an International Symposium*. New York: Alan R Liss; 1983:57.
8. Yuill TM. The role of mammals in the maintenance and dissemination of La Crosse virus. In: Calisher CH, Thompson WH, eds. *California Serogroup Viruses, Proceedings of an International Symposium*. New York: Alan R Liss; 1983:77.
9. Watts DM, Thompson WH, Yuill TM, et al. Overwintering of La Crosse virus in *Aedes triseriatus*. *Am J Trop Med Hyg*. 1974;23:694.
10. McJunkin JE, Khan RR, Tsai TF. California-La Crosse encephalitis. *Infect Dis Clin North Am*. 1998;12:83.
11. Jones TF, Erwin PC, Craig AS, et al. Serological survey and active surveillance for La Crosse virus infections among children in Tennessee. *Clin Infect Dis*. 2000;31: 1284–1287.
12. Centers for Disease Control and Prevention. Possible congenital infection with La Crosse encephalitis virus—West Virginia, 2006-2007. *MMWR Morb Mortal Wkly Rep*. 2009;58:4–7.
13. Cully JF, Streit TG, Geard PB. Transmission of La Crosse virus by four strains of *Aedes albopictus* and from the eastern chipmunk *(Tamias striatus)*. *J Am Mosq Control Assoc*. 1992;8:237.
14. Gerhardt RR, Gottfried KL, Apperson CS, et al. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerg Infect Dis*. 2001;7:807–811.
15. Eldridge BF, Glaser C, Pedrin RE, et al. The first reported case of California encephalitis in more than 50 years. *Emerg Infect Dis*. 2001;7:451–452.
16. Huang CW, Campbell L, Grady I, et al. Diagnosis of Jamestown Canyon encephalitis by polymerase chain reaction. *Clin Infect Dis*. 1999;28:1294–1297.
17. Lithicum KJ, Davies FG, Kairo A, et al. Rift Valley fever virus (family Bunyaviridae, genus *Phlebovirus*). Isolation from Diptera collected during an interepizootic period in Kenya. *J Hyg (Lond)*. 1985;95:197.
18. Swanepoel R, Coetzer JAW. Rift Valley fever. In: Coetzer JAW, Tustin RC, eds. *Infectious Diseases of Livestock*. 2nd ed. Oxford: Oxford University Press; 2004:1037–1070.
19. Woods CW, Karpati AM, Grein T, et al. An outbreak of Rift Valley fever in Northeastern Kenya, 1997-98. *Emerg Infect Dis*. 2002;8:138–144.
20. Fagbo SF. The evolving transmission pattern of Rift Valley fever in the Arabian Peninsula. *Ann N Y Acad Sci*. 2002; 969:201–204.
21. Bird BH, Githinji JW, Macharia JM, et al. Multiple virus lineages sharing recent common ancestry were associated with a large Rift Valley fever outbreak among livestock in Kenya during 2006-2007. *J Virol*. 2008;82:11152–11166.
22. Peters CJ. Emergence of Rift Valley fever. In: Saluzzo JF, Dodet B, eds. *Factors in the Emergence of Arbovirus Diseases*. Paris: Elsevier; 1997:253.
23. van Eeden PJ, Joubert JR, van de Wal BW, et al. A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part I. Clinical features. *S Afr Med J*. 1985;68:711–717.
24. Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2004;64:145–160.
25. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J Med Entomol*. 1979;15:307–417.
26. Leblebicioglu H, Ozaras R, Sunbul M. Crimean-Congo hemorrhagic fever: a neglected infectious disease with potential nosocomial infection threat. *Am J Infect Control*. 2017;45:815–816.
27. Ince Y, Yasa C, Metin M, et al. Crimean-Congo hemorrhagic fever infections reported by ProMED. *Int J Infect Dis*. 2014;26:44–46.
28. Leblebicioglu H, Ozaras R, Erciyas-Yavuz K. Emergence of Crimean-Congo hemorrhagic fever. *Trans R Soc Trop Med Hyg*. 2015;109:676–678.
29. Peters CJ, Mills JN, Spiropoulou C, et al. Hantavirus infections. In: Guerrant RL, Walker DH, Weller PF, eds. *Tropical Infectious Diseases—Principles, Pathogens and Practice*. 2nd ed. Philadelphia: Churchill Livingstone; 2006:762–780.
30. Lee HW, Lee PW, Baek LJ, et al. Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent *Apodemus agrarius*. *Am J Trop Med Hyg*. 1981;30:1106.
31. Hutchinson KL, Rollin PE, Peters CJ. Pathogenesis of a North American hantavirus, Black Creek Canal virus, in experimentally infected *Sigmodon hispidus*. *Am J Trop Med Hyg*. 1998;59:58.
32. Korpela H, Lahdevirta J. The role of small rodents and patterns of living in the epidemiology of nephropathia epidemica. *Scand J Infect Dis*. 1978;10:303.
33. Niklasson B, Hornfeldt B, Lindkvist A, et al. Temporal dynamics of Puumala virus antibody prevalence in voles and of nephropathia epidemica incidence in humans. *Am J Trop Med Hyg*. 1995;53:134.
34. Peters CJ. Hantavirus pulmonary syndrome in the Americas. In: Scheld WM, Craig WA, Hughes JM, eds. *Emerging Infections II*. Washington, DC: American Society for Microbiology Press; 1998:7–64.
35. Mather TN, DeFoliart GR. Dispersion of gravid *Aedes triseriatus* (Diptera: Culicidae) from woodlands into open terrain. *J Med Entomol*. 1984;21:384.
36. Rodriguez LL, Maupin GO, Ksiazek TG, et al. Molecular investigation of a multisource outbreak of Crimean-Congo hemorrhagic fever in the United Arab Emirates. *Am J Trop Med Hyg*. 1997;57:512.
37. Burney MI, Ghafoor A, Saleen M, et al. Nosocomial outbreak of viral hemorrhagic fever—Congo virus in Pakistan, January 1976. *Am J Trop Med Hyg*. 1980;29:941.
38. Deleted in review.
39. Papa A, Bino S, Llagami A, et al. Crimean-Congo hemorrhagic fever in Albania, 2001. *Eur J Clin Microbiol Infect Dis*. 2002;21:603–606.
40. Sunbul M, Leblebicioglu H, Fletcher TE, et al. Crimean-Congo hemorrhagic fever and secondary bacteremia in Turkey. *J Infect*. 2015;71:597–599.
41. Leblebicioglu H, Sunbul M, Guner R, et al. Healthcare-associated Crimean-Congo haemorrhagic fever in Turkey, 2002-2014: a multicentre retrospective cross-sectional study. *Clin Microbiol Infect*. 2016;22:387, e1–e4.
42. Pshenichnaya NY, Nenadskaya SA. Probable Crimean-Congo hemorrhagic fever virus transmission occurred after aerosol-generating medical procedures in Russia: nosocomial cluster. *Int J Infect Dis*. 2015;33:120–122.
43. Kawamata J, Yamanouchi T, Dohmae K, et al. Control of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) in Japan. *Lab Anim Sci*. 1987;37:431.
44. Hjelle B, Glass GE. Outbreak of hantavirus infection in the Four Corners region of the United States in the wake of the 1997-1998 El Niño-southern oscillation. *J Infect Dis*. 2000;181:1569–1573.
45. Chen HX, Qiu FX, Dong BJ, et al. Epidemiologic studies in hemorrhagic fever with renal syndrome in China. *J Infect Dis*. 1986;154:394.
46. McJunkin JE, de los Reyes EC, Irazuzta JE, et al. La Crosse encephalitis in children. *N Engl J Med*. 2001;344: 801–807.
47. Wurtz R, Paleologos N. La Crosse encephalitis presenting like herpes simplex encephalitis in an immunocompromised adult. *Clin Infect Dis*. 2000;31:1113–1114.
48. Deering WM. Neurological aspects and treatment of La Crosse encephalitis. In: Calisher CH, Thompson WH, eds. *California Serogroup Viruses. Proceedings of an International Symposium*. New York: Alan R Liss; 1983:187.
49. de los Reyes EC, McJunkin JE, Glauser TA, et al. Periodic lateralized epileptiform discharges in La Crosse encephalitis, a worrisome subgroup: clinical presentation, electroencephalogram (EEG) patterns, and long-term neurologic outcome. *J Child Neurol*. 2008;23:167–172.
50. Tsai TF. Congenital arboviral infections: something new, something old. *Pediatrics*. 2006;117:936–939.
51. Osorio JE, Schoepp RJ, Yuill TM. Effects of La Crosse infection on pregnant domestic rabbits and Mongolian gerbils. *Am J Trop Med Hyg*. 1996;55:384–390.
52. Edwards JF, Karabatsos N, Collisson EW, et al. Ovine fetal malformations induced by in utero inoculation with main drain, San Angelo, and La Crosse viruses. *Am J Trop Med Hyg*. 1997;56:171–176.
53. Bartelloni PJ, Tesh RB. Clinical and serologic responses of volunteers infected with phlebotomus fever virus (Sicilian type). *Am J Trop Med Hyg*. 1976;25:456–462.
54. Sabin AB. Experimental studies on *Phlebotomus* (pappataci, sandfly) fever during World War II. *Arch Gesamte Virusforsch*. 1951;4:367–410.
55. Al Hazmi M, Ayoola EA, Abdurahman M, et al. Epidemic Rift Valley fever in Saudi Arabia: a clinical study of severe illness in humans. *Clin Infect Dis*. 2003;36:245–252.
56. Mardani M, Keshtkar-Jahromi M. Crimean-Congo hemorrhagic fever. *Arch Iran Med*. 2007;10:204–214.
57. Ergonul O. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis*. 2006;6:203–214.
58. Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2004;64:145–160.
59. Lee JS, Cho BY, Lee MC, et al. Clinical features of serologically proven Korean hemorrhagic fever patients. *Seoul J Med*. 1980;21:163.
60. Earle DP. Symposium on epidemic hemorrhagic fever. *Am J Med*. 1954;16:617.
61. Bruno P, Harrison HL, Brown J, et al. The protean manifestations of hemorrhagic fever with renal syndrome: a retrospective review of 26 cases from Korea. *Ann Intern Med*. 1990;113:385.
62. Gavrilovskaya IN, Brown EJ, Ginsberg MH, et al. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. *J Virol*. 1999;73:3951–3959.
63. Gavrilovskaya IN, Peresleni T, Geimonen E, et al. Pathogenic hantaviruses selectively inhibit beta3 integrin directed endothelial cell migration. *Arch Virol*. 2002;147:1913–1931.
64. Peters CJ, Simpson G, Levy H. Spectrum of hantavirus infection: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. *Annu Rev Med*. 1999;50:531–545.
65. Taylor SL, Wahl-Jensen V, Copeland AM, et al. Endothelial cell permeability during hantavirus infection involves factor XII-dependent increased activation of the kallikrein-kinin system. *PLoS Pathog*. 2013;9:e1003470.
66. Antonen J, Leppänen I, Tenhunen J, et al. A severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant. *Scand J Infect Dis*. 2013;45:494.
67. Paakkala A, Mustonen J, Viander M, et al. Complement activation in nephropathia epidemica caused by Puumala hantavirus. *Clin Nephrol*. 2000;53:424.
68. Sane J, Laine O, Mäkelä S, et al. Complement activation in Puumala hantavirus infection correlates with disease severity. *Ann Med*. 2012;44:468.
69. Amara U, Flierl MA, Rittirsch D, et al. Molecular intercommunication between the complement and coagulation systems. *J Immunol*. 2010;185:5628.
70. Mäkelä S, Ala-Houhala I, Mustonen J, et al. Renal function and blood pressure five years after Puumala virus-induced nephropathy. *Kidney Int*. 2000;58:1711.
71. Miettinen MH, Mäkelä SM, Ala-Houhala IO, et al. Ten-year prognosis of Puumala hantavirus-induced acute interstitial nephritis. *Kidney Int*. 2006;69:2043.
72. Kim YS, Ahn C, Han JS, et al. Hemorrhagic fever with renal syndrome caused by the Seoul virus. *Nephron*. 1995;71:419–427.
73. Duchin JS, Koster F, Peters CJ, et al. Hantavirus pulmonary syndrome: clinical description of disease caused by a newly recognized hemorrhagic fever virus in the southwestern United States. *N Engl J Med*. 1994;330: 949.
74. Moolenaar RL, Dalton C, Lipman HB, et al. Clinical features that differentiate hantavirus pulmonary syndrome from three other acute respiratory illnesses. *Clin Infect Dis*. 1995;21:643.
75. Pergam SA, Schmidt DW, Nofchissey RA, et al. Potential renal sequelae in survivors of hantavirus cardiopulmonary syndrome. *Am J Trop Med Hyg*. 2009;80:279–285.
76. Peters CJ, Khan AS. Hantavirus pulmonary syndrome: the new American hemorrhagic fever. *Clin Infect Dis*. 2002;34:1224–1231.
77. Ketai LH, Williamson MR, Telepak RJ, et al. Hantavirus pulmonary syndrome: radiographic findings in 16 patients. *Radiology*. 1994;191:665.
78. Dietl CA, Wernly JA, Pett SB, et al. Extracorporeal membrane oxygenation support improves survival of patients with severe hantavirus cardiopulmonary syndrome. *J Thorac Cardiovasc Surg*. 2008;135:579–584.
79. Zaki SR, Greer PW, Coffield LM, et al. Hantavirus pulmonary syndrome: pathogenesis of an emerging infectious disease. *Am J Pathol*. 1995;146:552.
80. Calisher CH, Pretzman CI, Muth DJ, et al. Serodiagnosis of La Crosse virus infections in humans by detection of immunoglobulin M class antibodies. *J Clin Microbiol*. 1986;12:667.
81. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science*. 1998;281:1305–1308.
82. Swanepoel R. Crimean-Congo haemorrhagic fever. In: Palmer SR, Soulsby EJL, Simpson DIH, eds. *Zoonoses*. Oxford: Oxford University Press; 1998:461–470.
83. Nichol ST, Spiropoulou CF, Morzunov S, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science*. 1993;262:914–917.

84. Peters CJ, Reynolds JA, Slone TW, et al. Prophylaxis of Rift Valley fever with antiviral drugs, immune serum, an interferon inducer, and a macrophage activator. *Antiviral Res*. 1986;6:285.
85. Mardani M, Jahromi MK, Naieni KH, et al. The efficacy of oral ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Iran. *Clin Infect Dis*. 2003;36:1613–1618.
86. Johnson S, Henschke N, Maayan N, et al. Ribavirin for treating Crimean Congo haemorrhagic fever. *Cochrane Database Syst Rev*. 2018;(6):CD012713.
87. Ceylan B, Turhan V. The efficacy of ribavirin in Crimean-Congo hemorrhagic fever–randomized trials are urgently needed. *Int J Infect Dis*. 2014;29:297.
88. McJunkin JE, Khan R, de los Reyes EC, et al. Treatment of severe La Crosse encephalitis with intravenous ribavirin following diagnosis by brain biopsy. *Pediatrics*. 1997;99:261.
89. Vial PA, Valdivieso F, Calvo M, et al. A non-randomized multicentre trial of human immune plasma for treatment of hantavirus cardiopulmonary syndrome caused by Andes virus. *Antivir Ther*. 2015;20:377–386.
90. Francy DB. Mosquito control for prevention of California (La Crosse) encephalitis. In: Calisher CH, Thompson WH, eds. *California Serogroup Viruses. Proceedings of an International Symposium*. New York: Alan R Liss; 1983:365.
91. El-Karamany RM, Imam IZE, Farid AH, et al. Production of inactivated RVF vaccine. *J Egypt Pub Health Assoc*. 1981;56:495–525.
92. Coackley W, Pini A, Gosden D. The immunity induced in cattle and sheep by inoculation of neurotropic or pantropic Rift Valley fever viruses. *Res Vet Sci*. 1967;8:406–414.
93. Keshtkar-Jahromi M, Kuhn JH, Christova I, et al. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. *Antiviral Res*. 2011;90:85–92.
94. Buttigieg KR, Dowall SD, Findlay-Wilson S, et al. A novel vaccine against Crimean-Congo haemorrhagic fever protects 100% of animals against lethal challenge in a mouse model. *PLoS ONE*. 2014;9:e91516.
95. Hooper JW, Li D. Vaccines against hantaviruses. *Curr Top Microbiol Immunol*. 2001;256:171–191.
96. Pastula DM, Hoang Johnson DK, White JL, et al. Jamestown Canyon virus disease in the United States-2000-2013. *Am J Trop Med Hyg*. 2015;93:384–389.
97. Pastula DM, Smith DE, Beckham JD, et al. Four emerging arboviral diseases in North America: Jamestown Canyon, Powassan, chikungunya, and Zika virus diseases. *J Neurovirol*. 2016;22:257–260.
98. Centers for Disease Control and Prevention. CDC Arboviral Catalog. 2018. https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=206&SID=2. Accessed November 26, 2018.
99. Watts DM, Phillips I, Callahan JD, et al. Oropouche virus transmission in the Amazon River basin of Peru. *Am J Trop Med Hyg*. 1997;56:148.
100. Bernardes-Terzian AC, de-Moraes-Bronzoni RV, Drumond BP, et al. Sporadic oropouche virus infection, Acre, Brazil. *Emerg Infect Dis*. 2009;15:348–350.
101. Pinheiro FP, Travassos da Rosa AP, Travassos da Rosa JF, et al. Oropouche virus. I. A review of clinical, epidemiological, and ecological findings. *Am J Trop Med Hyg*. 1981;30:149.
102. Tesh RB. The emerging epidemiology of Venezuelan hemorrhagic fever and Oropouche fever in tropical South America. *Ann N Y Acad Sci*. 1994;740:129.
103. Mourãão MP, Bastos MS, Gimaqu JB, et al. Oropouche fever outbreak, Manaus, Brazil, 2007-2008. *Emerg Infect Dis*. 2009;15:2063.
104. Travassos da Rosa JF, de Souza WM, Pinheiro FP, et al. Oropouche virus: clinical, epidemiological, and molecular aspects of a neglected *Orthobunyavirus*. *Am J Trop Med Hyg*. 2017;96:1019.
105. Mourão MP, Bastos Mde S, Figueiredo RM, et al. Arboviral diseases in the Western Brazilian Amazon: a perspective and analysis from a tertiary health & research center in Manaus, State of Amazonas. *Rev Soc Bras Med Trop*. 2015;48(suppl 1):20.
106. World Health Organization. Oropouche Virus Disease—Peru. 2016. http://www.who.int/csr/don/03-june-2016-oropouche-peru/en/. Accessed August 16, 2016.
107. Nicoletti L, Ciufolini MG, Verani P. Sandfly fever viruses in Italy [review]. *Arch Virol Suppl*. 1996;11:41.
108. Nicoletti L, Verani P, Caciolli S, et al. Central nervous system involvement during infection by *Phlebovirus* Toscana of residents in natural foci in central Italy (1977-1988). *Am J Trop Med Hyg*. 1991;45:429.
109. Charrel RN, Gallian P, Navarro-Mari JM, et al. Emergence of Toscana virus in Europe. *Emerg Infect Dis*. 2005;11:1657.
110. Di Nicuolo G, Pagliano P, Battisti S, et al. Toscana virus central nervous system infections in southern Italy. *J Clin Microbiol*. 2005;43:6186.
111. Braito A, Ciufolini MG, Pippi L, et al. *Phlebotomus*-transmitted Toscana virus infections of the central nervous system: a seven-year experience in Tuscany. *Scand J Infect Dis*. 1998;30:505–508.
112. Echevarria JM, de Ory F, Guisasola ME, et al. Acute meningitis due to Toscana virus infection among patients from both the Spanish Mediterranean region and the region of Madrid. *J Clin Virol*. 2003;26:79–84.
113. Pierro A, Ficarelli S, Ayhan N, et al. Characterization of antibody response in neuroinvasive infection caused by Toscana virus. *Clin Microbiol Infect*. 2017;23:868–873.
114. Gerrard SR, Li L, Barrett AD, et al. Ngari virus is a Bunyamwera virus reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa. *J Virol*. 2004;78:8922–8926.
115. Groseth A, Weisend C, Ebihara H. Complete genome sequencing of mosquito and human isolates of Ngari virus. *J Virol*. 2012;86:13846–13847.
116. Odhiambo C, Venter M, Lwande O, et al. Phylogenetic analysis of Bunyamwera and Ngari viruses (family Bunyaviridae, genus *Orthobunyavirus*) isolated in Kenya. *Epidemiol Infect*. 2016;144:389–395.
117. Bowen MD, Trappier SG, Sanchez AJ, et al. A reassortant bunyavirus isolated from acute hemorrhagic fever cases in Kenya and Somalia. *Virology*. 2001;291:185–190.
118. Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. *N Engl J Med*. 2011;364:1523–1532.
119. Zhang L, Liu W, Ni D, et al. Nosocomial transmission of human granulocytic anaplasmosis in China. *JAMA*. 2008;300:2263–2270.
120. Bao CJ, Guo XL, Qi X, et al. A family cluster of infections by a newly recognized bunyavirus in eastern China, 2007: further evidence of person-to-person transmission. *Clin Infect Dis*. 2011;53:1208–1214.
121. Gai ZT, Zhang Y, Liang MF, et al. Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. *J Infect Dis*. 2012;206:1095–1102.
122. Liu Y, Li Q, Hu W, et al. Person-to-person transmission of severe fever with thrombocytopenia syndrome virus. *Vector Borne Zoonotic Dis*. 2012;12:156–160.
123. Kim WY, Choi W, Park SW, et al. Nosocomial transmission of severe fever with thrombocytopenia syndrome in Korea. *Clin Infect Dis*. 2015;60:1681–1683.
124. Odhiambo C, Venter M, Lwande O, et al. Phylogenetic analysis of Bunyamwera and Ngari viruses (family Bunyaviridae, genus *Orthobunyavirus*) isolated in Kenya. *Epidemiol Infect*. 2016;144:389–395.
125. Liu Q, He B, Huang SY, et al. Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis. *Lancet Infect Dis*. 2014;14:763.
126. Takahashi T, Maeda K, Suzuki T, et al. The first identification and retrospective study of Severe Fever with Thrombocytopenia Syndrome in Japan. *J Infect Dis*. 2014;209:816.
127. Zhang YZ, He YW, Dai YA, et al. Hemorrhagic fever caused by a novel Bunyavirus in China: pathogenesis and correlates of fatal outcome. *Clin Infect Dis*. 2012;54:527.
128. Gai ZT, Zhang Y, Liang MF, et al. Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. *J Infect Dis*. 2012;206:1095.
129. Kato H, Yamagishi T, Shimada T, et al. Epidemiological and clinical features of severe fever with thrombocytopenia syndrome in Japan, 2013-2014. *PLoS ONE*. 2016;11:e0165207.
130. Guo CT, Lu QB, Ding SJ, et al. Epidemiological and clinical characteristics of severe fever with thrombocytopenia syndrome (SFTS) in China: an integrated data analysis. *Epidemiol Infect*. 2016;144:1345.
131. Sun J, Lu L, Wu H, et al. The changing epidemiological characteristics of severe fever with thrombocytopenia syndrome in China, 2011-2016. *Sci Rep*. 2017;7:9236.
132. Choi SJ, Park SW, Bae IG, et al. Severe fever with thrombocytopenia syndrome in South Korea, 2013-2015. *PLoS Negl Trop Dis*. 2016;10:e0005264.
133. Deng B, Zhang S, Geng Y, et al. Cytokine and chemokine levels in patients with severe fever with thrombocytopenia syndrome virus. *PLoS ONE*. 2012;7:e41365.
134. Sun Y, Jin C, Zhan F, et al. Host cytokine storm is associated with disease severity of severe fever with thrombocytopenia syndrome. *J Infect Dis*. 2012;206:1085.
135. Hu LF, Wu T, Wang B, et al. The regulation of seventeen inflammatory mediators are associated with patient outcomes in severe fever with thrombocytopenia syndrome. *Sci Rep*. 2018;8:159.
136. Sun Y, Liang M, Qu J, et al. Early diagnosis of novel SFTS bunyavirus infection by quantitative real-time RT-PCR assay. *J Clin Virol*. 2012;53:48.
137. McMullan LK, Folk SM, Kelly AJ, et al. A new phlebovirus associated with severe febrile illness in Missouri. *N Engl J Med*. 2012;367:834–841.
138. Deleted in review.
139. Centers for Disease Control and Prevention. Heartland Virus Disease. https://www.cdc.gov/ticks/tickbornediseases/heartland-virus.html. Accessed November 29, 2018.
140. Savage HM, Godsey MS Jr, Lambert A, et al. First detection of heartland virus (Bunyaviridae: *Phlebovirus*) from field collected arthropods. *Am J Trop Med Hyg*. 2013;89:445.
141. Riemersma KK, Komar N. Heartland virus neutralizing antibodies in vertebrate wildlife, United States, 2009-2014. *Emerg Infect Dis*. 2015;21:1830.
142. Bosco-Lauth AM, Panella NA, Root JJ, et al. Serological investigation of heartland virus (Bunyaviridae: *Phlebovirus*) exposure in wild and domestic animals adjacent to human case sites in Missouri 2012-2013. *Am J Trop Med Hyg*. 2015;92:1163.
143. Fill MA, Compton ML, McDonald EC, et al. Novel clinical and pathologic findings in a Heartland virus-associated death. *Clin Infect Dis*. 2017;64:510.
144. Muehlenbachs A, Fata CR, Lambert AJ, et al. Heartland virus-associated death in Tennessee. *Clin Infect Dis*. 2014;59:845.
145. Oklahoma State Department of Health. Oklahoma State Health Department Confirms First Case and Death of Heartland Virus, May 24, 2014. https://www.ok.gov/health/Organization/Office_of_Communications/News_Releases/2014_News_Releases/Oklahoma_State_Health_Department_Confirms_First_Case_and_Death_of_Heartland_Virus.html. Accessed November 29, 2018.
146. Pastula DM, Turabelidze G, Yates KF, et al. Notes from the field: Heartland virus disease - United States, 2012-2013. *MMWR Morb Mortal Wkly Rep*. 2014;63:270.
147. Carlson AL, Pastula DM, Lambert AJ, et al. Heartland virus and hemophagocytic lymphohistiocytosis in immunocompromised patient, Missouri, USA. *Emerg Infect Dis*. 2018;24:893–897.
148. Centers for Disease Control and Prevention. Clinical and Lab Evaluations, Heartland Virus. https://www.cdc.gov/heartland-virus/healthcare-providers/clinical-lab-evals.html. Accessed November 29, 2018.

167 Lymphocytic Choriomeningitis Virus, Lassa Virus, and the South American Hemorrhagic Fevers (Arenaviruses)

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SHORT VIEW SUMMARY

Epidemiology
- Lymphocytic choriomeningitis virus (LCMV) is worldwide in distribution, Lassa fever is found in Africa, and the New World complex viruses circulate in South and North America and can cause hemorrhagic fevers.
- Transmission is by rodents, with generally high species specificity of reservoir vectors.

Microbiology
- These pleomorphic viruses are 50 to 300 nm in diameter with two single RNA segments with ambisense gene organization.

Clinical Manifestations
- *LCMV:* Febrile illness is accompanied by headache and systemic symptoms; the disease course may be biphasic with meningitis manifesting during the second phase in 5 to 10 days; full recovery is usual, but immunosuppression may result in disseminated and fatal disease.
- *Lassa fever:* The illness is usually mild, but severe multisystem disease may develop in 5% to 10% of cases, resulting in death in 15% to 25% of hospitalized patients; fever, pharyngitis, retrosternal pain, and proteinuria are the most common features; fatal cases usually occur in the second week of illness, with hypotension, vasoconstriction, mucosal hemorrhages, and capillary leak syndrome.
- *South American hemorrhagic fevers:* Argentinean, Bolivian, and Venezuelan hemorrhagic fevers have progressive fever, malaise, and myalgias, most frequently affecting the lower back; petechiae and small vesicles are frequent; vascular disease (capillary leak), vasoconstriction, and shock may occur; various blood and neurologic abnormalities can be seen; overall mortality is 15% to 30%; convalescence may take weeks, but recovery is usually without sequelae.

Diagnosis
- Detection of virus is evident in acute blood specimens by reverse-transcriptase polymerase chain reaction assay or virus culture, depending on the particular viral agent.
- Immunoglobulin M antibodies may be detected by serology, and increases in antibody titers (seroconversion) can be noted by comparing acute and convalescent serum specimens.

Treatment
- Supportive treatment may be lifesaving.
- Convalescent human plasma has been shown to be effective in Argentinean hemorrhagic fever but has not been as successful in Lassa fever.
- Ribavirin is beneficial in treatment of patients hospitalized with Lassa fever and in patients with Argentinean and Bolivian hemorrhagic fevers.

Prevention
- Reduction of transmission from rodents to humans is needed.
- Person-to-person transmission in hospitals is a problem with Lassa fever, and use of gloves and gowns and careful disposal of patients' wastes and fomites should be implemented; if available, single rooms with negative pressure should be used.
- Steps should be taken to avoid infection in laboratory workers who examine samples that may contain arenaviruses.
- A live-attenuated vaccine is available against Argentinean hemorrhagic fever and is licensed in Argentina.

Arenaviruses are transmitted by rodents and comprise several medically important pathogens that cause hemorrhagic fevers in humans (Table 167.1). All arenaviruses can be divided based on their phylogenetic and antigenic relations into the Old World and New World virus complexes. The Old World complex includes arenaviruses that circulate worldwide and in Africa (i.e., lymphocytic choriomeningitis [LCMV] and Lassa [LASV] viruses). The New World complex includes viruses that circulate in South and North America (i.e., Junin [JUNV], Machupo [MACV], Guanarito [GTOV], and Sabia [SABV] viruses) and is further subdivided into three distinct clades designated as A, B, and C. The prototypic arenavirus LCMV has a worldwide distribution due to its association with *Mus domesticus* and *Mus musculus* and can cause febrile disease sometimes with central nervous system involvement as well as congenital infections. LASV is enzootic among rodents of the Murinae subfamily of the family Muridae inhabiting sub-Saharan Africa and is a causative agent of Lassa fever (LF), a severe disease in humans. There are at least four pathogenic South American arenaviruses, JUNV, MACV, GTOV, and SABV, that are transmitted by rodents of the Sigmodontinae subfamily of the family Muridae and are known to cause hemorrhagic fever in humans in endemic areas of Argentina, Bolivia, Venezuela, and Brazil.

VIRAL CHARACTERIZATION

Arenaviruses (family Arenaviridae) have pleomorphic virions ranging in diameter from 50 to 300 nm with an average diameter about 110 to 130 nm.[1] Electron micrographs reveal a granular appearance of the virion interior that is characteristic for all arenaviruses and is attributed to the presence of the host cell ribosomes (Fig. 167.1). This appearance gave the name to this family of viruses (Latin term *arenosus* means "sandy").

The genome of arenaviruses consists of two single-stranded RNA segments, large (L) and small (S). Each segment uses an ambisense gene organization to drive expression of two genes in opposite directions. The L RNA segment (approximately 7.1 kb) encodes the viral RNA–dependent RNA polymerase (L) and a small RING finger protein (Z) that is the arenavirus counterpart of the matrix proteins (M) of negative-sense RNA viruses. The S RNA segment (approximately 3.4 kb) encodes the glycoprotein precursor protein and the nucleoprotein (NP). Glycoprotein precursor protein is posttranslationally cleaved to yield two envelope glycoproteins, GP1 and GP2, and the stable signal peptide, which stays associated with the glycoprotein complex on mature virions.

The 250-kDa L polymerase uses the viral ribonucleoprotein consisting of genome RNA encapsidated by NP as a template to synthesize

TABLE 167.1 Arenaviruses and Human Disease

VIRUS	DISEASE	GEOGRAPHY	RESERVOIR	PATHOGENESIS	SPECIFIC THERAPY	PREVENTION
Lymphocytic choriomeningitis	Aseptic meningitis; other organ involvement	North and South America, Europe, and wherever *Mus* is introduced	*Mus domesticus* and *Mus musculus* (house mice)	Systemic infection; when CNS invasion occurs, immunopathologic CNS disease follows	None	House mouse control and avoidance, particularly by pregnant women; monitor mouse and hamster suppliers
Lassa	Lassa fever	West Africa, particularly Sierra Leone, Guinea, Liberia, and Nigeria	*Mastomys* (multimammate mouse)	Vascular leak, multiorgan dysfunction, shock; bleeding and CNS involvement occur but not as common as in South American diseases	Intravenous ribavirin	Rodent avoidance and control in houses may be of ancillary benefit; strict isolation of hospitalized patients
Junin	Argentinean HF	Argentine pampas	*Calomys musculinis*	As Lassa fever except encephalopathy and thrombocytopenia are common, as is hemorrhage	Convalescent plasma; ribavirin probably efficacious	Effective live-attenuated vaccine
Machupo	Bolivian HF	Bolivia (Beni Department)	*Calomys callosus*	As Argentinean HF	Ribavirin or convalescent plasma	Elimination of rodents from home; laboratory evidence for cross-protection by Junin vaccine
Chapare	Chapare HF	Bolivia (Cochabamba Department)	Unknown	Unknown, resembles Argentinean HF or Sabia infection	Unknown; ribavirin suggested	Unknown
Guanarito	Venezuelan HF	Venezuela (Portuguesa State)	*Zygodontomys brevicauda*	As Argentinean HF	Unknown; ribavirin or convalescent plasma suggested	Unknown; rodent control?
Sabia	Brazilian HF	Brazil	Unknown	Resembles Argentinean HF; a patient in single naturally occurring case had severe hepatitis	Unknown; ribavirin suggested	Unknown

CNS, Central nervous system; *HF,* hemorrhagic fever.

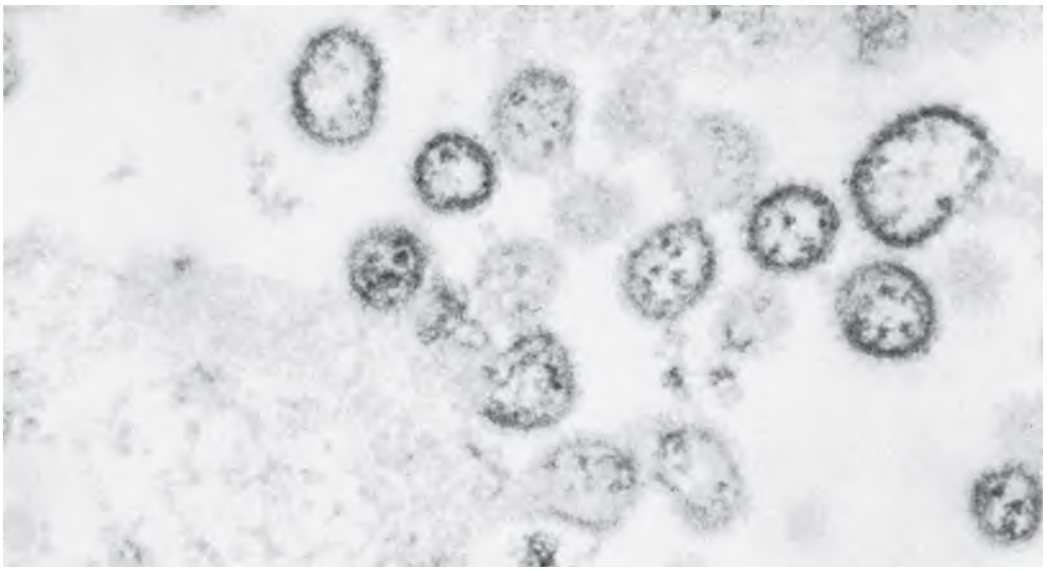

FIG. 167.1 Lassa virus. Electron micrograph of Lassa virus in the first Vero cell passage envelope; electron-dense interior granules can be seen (original magnification, ×121,000).

antigenomic copies of both RNA segments and capped nonpolyadenylated messenger RNA (mRNA) of the four viral genes.[2] Synthesis of viral RNA is initiated from the promoter regions located at the 3′-end of genomic and antigenomic segment RNAs. Viral transcription terminates at the distal side of the stem-loop structures within the intergenic regions that separate viral genes on both L and S RNA segments. These stem-loop structures have been proposed to stabilize the 3′-termini of the viral mRNAs.[3,4] Arenaviruses acquire cap structures of host cell mRNA by a cap-snatching mechanism mediated by the cap-binding activity of NP and endonuclease activity of the L polymerase.[5–7] The matrix protein Z is not required for the activity of the viral replication complex. On the contrary, Z has a dose-dependent inhibitory effect on viral RNA synthesis by locking the L polymerase in a promoter-bound state. This function of Z has been proposed to contribute to the switch between

active expression and replication of the viral genome and virion packaging, ensuring inclusion of the L polymerase into the maturing viral particles.[8–10] This inhibitory effect of Z is highly conserved throughout the *Arenaviridae* family, as it has been demonstrated for both New World[11] and Old World[12] arenaviruses.

Old World and clade C New World arenaviruses use α-dystroglycan, a cellular receptor for proteins of the extracellular matrix, to direct entry into cells.[13] Transferrin receptor 1 (TfR1) is the primary receptor used by the glycoprotein complexes of pathogenic New World arenaviruses; however, cell entry of nonpathogenic viruses is TfR1 independent. Glycoproteins of pathogenic members of the New World group can also use TfR1-independent entry pathways, although less efficiently.[14]

In addition to the functions required to support virus replication and formation of infectious progeny, viral proteins are also involved in the modulation of the host cell response to infection. Thus NP has been demonstrated to contribute to virus-induced inhibition of type I interferon signaling, which has been attributed to the 3′–5′ exoribonuclease activity mapped to the C-terminal domain of NP.[5,15–18] Besides being the main driving force of arenavirus budding and an essential regulator of the viral replication complex, the matrix protein Z has been shown to interact with a number of cellular factors and contribute to repression of cellular translation, evasion of infection-induced apoptosis, and inhibition of type I interferon induction.[19–21]

EPIDEMIOLOGY

Arenaviruses are transmitted by rodents and have high species specificity, with one species usually being the reservoir for one given virus. There are three possible outcomes for infected animals: rapid clearance of virus, development of an acute lethal disease, and persistent inapparent infection with the release of virus into excreta, specifically urine, which is believed to be the main cause of human infections through inhalation of aerosolized viral particles. Both vertical transmission and horizontal spread contribute to the maintenance of virus in the rodent population.

Lymphocytic Choriomeningitis

Although LCMV has potential for worldwide distribution, human cases have been reported and documented only in Europe and the Americas. Moreover, the prevalence of LCMV infection in both nonoverlapping reservoir species, *Mus domesticus* and *Mus Muscullus,* is highly focal, as was evident from surveillance studies conducted in various areas of the United States and Germany.[22] Thus distribution of mice that tested positive for antibody to LCMV in Baltimore, Maryland, and Washington, DC, was found to be significantly clustered within blocks and even households.[23] Similarly, much higher LCMV infection rates among mice were detected in west-central Germany than in the southern or northern parts of the country.[24] This phenomenon can be explained by the highly structured social system of mice, which promotes inbreeding and therefore limits dispersal.

Human cases of lymphocytic choriomeningitis (LCM) most commonly occur in autumn. This pattern of infection activity is associated with seasonal increase of rodent population density and migration of mice into homes and barns caused by cold weather. Additionally, greater stability of LASV virions was demonstrated at low relative humidity and moderate temperatures in the natural environment.[25] Situations associated with wild mouse infection of humans include substandard housing such as mobile homes or inner-city dwellings, the cleaning of rodent-infested barns or outbuildings, and the autumn entry of wild mice into dwellings. Most human infections occur among young adults, likely due to increased professional and personal activities associated with the maintenance of houses and surrounding properties, although people of all ages have been affected. The exact route of transmission of LCMV from infected mice to humans in still not known. Epidemiologic and experimental data suggest aerosolized virions, direct contact, and bites of infected rodents as most likely causes of human infections.[26–28]

The incubation period for LCM in humans ranges from 5 to 10 days. The disease begins as a febrile illness that in severe cases can progress to acute meningitis in 2 to 3 weeks after exposure. Most sporadic LCM cases have been attributed to the handling of laboratory mice or Syrian hamsters; however, several human exposures resulted from contact with hamsters injected with LCMV-infected tumor cell lines.[28] In addition, an epidemic of laboratory-acquired LCM resulted from handling of nude, athymic mice infected through hamster tumor cell lines.[29] Other outbreaks in the United States and Europe resulted from exposure to pet hamsters obtained from breeders with infected stocks.[30,31] LCM has also been associated with organ transplantation as discussed in "Clinical Manifestations."

Lassa Fever

LASV was first isolated in 1969 from a missionary nurse who worked in a clinic in a small town, Lassa, in northeastern Nigeria.[32] Presumably the nurse acquired infection from an obstetric patient who lived in Lassa. The nurse died approximately 1 week after the onset of clinical symptoms. Subsequently, two more nurses who provided care to the first nurse contracted the disease, which resulted in the death of one of them. Virus isolates were recovered in cultured cells from all three cases, and the disease was named Lassa fever.[33]

Several countries in West Africa were initially identified as endemic for LASV including Sierra Leone,[34,35] Guinea,[36,37] Liberia,[38–40] and Nigeria.[41–43] However, after a serologic survey conducted among hospitalized patients with fever and missionaries with a history of a febrile illness, LASV was concluded to be also present in Ivory Coast, Mali, and Central African Republic.[44] The results of an investigation of a case of LF imported to Germany that revealed the index patient had traveled during the incubation period through Ghana, Ivory Coast, and Burkina Faso, further supported the notion that LASV was endemic in larger areas of West Africa.[45] Later, LASV was demonstrated to circulate in Burkina Faso, Ivory Coast, Ghana, Senegal, Gambia, and Mali.[46,47]

The multimammate mouse, *Mastomys natalensis,* was originally identified as the primary reservoir for LASV.[48] Moreover, newborn *M. natalensis* mice infected intraperitoneally develop persistent asymptomatic infection, which suggests the importance of this species for the maintenance of the virus in nature.[49] However, because the taxonomy of the genus *Mastomys* is poorly understood, it still is uncertain which species and particular subspecies can serve as a host for LASV.[50]

LASV has been estimated to cause 100,000 to 300,000 infections and approximately 5000 deaths each year.[51] However, given that up to 55% of the general population living in endemic areas test positive for the presence of LASV-specific antibodies, most infections probably result in a mild disease or are even asymptomatic and do not require hospitalization.[34,36,42,52] This is also supported by the high incidence of seroconversion, from 5% to 20%, of the nonimmune population per year.[34] Infection with LASV presumably occurs through either inhalation of aerosols or contact with body fluids or excreta produced by infected animals. The viral particles are stable in aerosol, and horizontal animal-to-animal transmission has been demonstrated experimentally.[25,53] Hunting and consumption of meat of peridomestic rodents have also been suggested to be an important route of LASV transmission to humans.[37]

The incubation period of LF ranges from 7 to 21 days.[32] The clinical disease begins as a flulike illness characterized by fever, general weakness, and malaise, which may be accompanied by cough, sore throat, and severe headache. Common complications include gastrointestinal manifestations such as nausea, vomiting, and diarrhea.[54] High prevalence of many other acute diseases in West Africa can significantly complicate differential diagnosis of LF based on presenting symptoms.[54,55] Hemorrhagic manifestations may not always be present in LF; however, perturbation of vascular function is likely to play an essential role in LASV infection–induced pathologic changes. In severe cases, the condition of the patient rapidly deteriorates between the 6th and 10th day of illness with severe pulmonary edema; acute respiratory distress; clinical signs of encephalopathy, sometimes with coma and seizures; and terminal shock.[54] Recovery from LF generally begins 8 to 10 days after disease onset. Sensorineural deafness, a common neurologic manifestation, can be observed in the late stages of the disease or in early convalescence in survivors.[56]

Other African Arenaviruses

Several other Old World arenaviruses circulate in Africa; however, none of them have been demonstrated to be pathogenic in humans. A safari-booking agent in Zambia contracted a disease that resembled a viral hemorrhagic fever. The index patient and three secondary cases died

of the disease. A single tertiary case was administered ribavirin and survived. The isolated virus has been identified as a new arenavirus with potentially high case-fatality rate and named Lujo after the geographic locations where the infection was discovered and investigated (Lusaka, Zambia, and Johannesburg, South Africa).[57]

South American Hemorrhagic Fevers

Viral hemorrhagic fevers caused by the arenaviruses JUNV, MACV, and GTOV that circulate in South America pose serious public health problems in Argentina, Bolivia, and Venezuela. Another arenavirus that can induce a lethal disease in humans, SABV, was discovered in Brazil in 1990; however, its rodent reservoir has not been identified, and its impact on public health is currently unclear.[58] A sudden appearance of a new viral hemorrhagic fever was detected in December 2003–January 2004 near Cochabamba, Bolivia, 400 km away from the epicenter of MACV activity. The virus isolated from the specimens collected from a fatal case was named Chapare. However, the characterization of this viral agent is still incomplete and missing vital pieces of information such as the natural rodent host and well-defined epidemiology and clinical picture.[58]

JUNV, the causative agent of Argentinian hemorrhagic fever (AHF), causes chronic infections of rodents that belong to the species *Calomys musculinus*.[59] AHF initially occurred in the region of humid pampas of northern Buenos Aires province and then spread to a larger area encompassing two more provinces and putting 5 million people at risk. The population of reservoir rodents in cornfields peaks between February and May, and therefore the disease primarily affects men engaged in harvesting corn. Human infections are believed to occur through either inhalation of aerosolized viral particles, which is considered the most common route of transmission, or direct contact of abraded skin with blood and tissues of infected rodents crushed by agricultural machinery. Person-to-person transmission is very rare and may occur via direct contact with body fluids of a viremic patient; nosocomial infections have also been reported.[60] Before the introduction of a specific vaccine, approximately 200 to 2000 cases of AHF were reported annually, and mechanization of the harvesting process did not result in a substantial decrease of AFH cases. Development of a live-attenuated vaccine, Candid 1, by the US Army Medical Research Institute of Infectious Diseases led to a significant reduction in the incidence of AFH and reduced the number of reported cases to less than 100 per year.[61]

Circulation of MACV, the causative agent of Bolivian hemorrhagic fever (BHF), is restricted to the tropical savanna of the Beni Department in the northeastern part of Bolivia. The large vesper mouse, *Calomys callosus,* is the rodent host for MACV. In this region the mice can freely enter houses, and human exposure is believed to occur by aerosols from infected animals or consumption of food contaminated with urine of infected rodents. The incidence of infection is greatest from the late rainy (April) to the early dry (July) seasons. The hallmark feature of BHF is that occurrence of an epidemic is usually followed by several years of quiescence. In town epidemics, where people of all ages and both sexes are equally exposed, the disease equally affects all these categories. On remote ranches and in fields, men (usually agricultural workers) are a predominant target. Person-to-person as well as nosocomial infections have been reported; however, this route of transmission is not common.[62,63] For both AHF and BHF, the incidence of human infections and horizontal spread of JUNV and MACV among reservoir rodents correlate with the fluctuations in the rodent population density.[64]

In 1989 in the Guanarito municipality of Portuguesa State and adjacent regions of Barinas State of Venezuela, human cases of a new viral hemorrhagic fever, Venezuelan hemorrhagic fever, were discovered. The new virus was named Guanarito (GTOV) after the geographic location where most human cases were detected. The cane mouse *Zygodontomys brevauda* was identified as the reservoir. Venezuelan hemorrhagic fever also has a cyclic behavior, and epidemic periods of high incidence occur every 4 to 5 years mainly affecting male agricultural workers between 14 and 54 years of age.[65]

Based on clinical observations of patients with AHF and BHF, the incubation period after natural exposure to South American arenaviruses has been determined to range from 5 to 19 days, with a median of 7 to 12 days. The incubation period of South American hemorrhagic fevers can be significantly shortened after parenteral infections, and the onset of symptoms can begin 2 to 6 days after virus exposure.

Other Arenaviruses

Several other arenaviruses related to LASV and LCMV and belonging to the Old World group have been discovered, such as Mopeia, Mobala, and Ippy, but no human disease has been found to be associated with these viral agents. Moreover, it is unclear if these arenaviruses can productively, if at all, infect humans. In addition, these viruses are attenuated in the two most relevant to human disease animal models of infection, nonhuman primates and guinea pigs.[26,53] Similarly, there are many arenaviruses that circulate in both Americas and belong to the New World group (this group is larger in general) that have not been described as human pathogens.[2] However, some of these viruses have caused infections of laboratory personnel without inducing disease (Pichinde virus), and many are currently being monitored, for example, Whitewater Arroyo virus, which is believed to have caused fatal human disease in the western United States.[66]

PATHOGENESIS

Rodents

Arenaviruses can cause chronic infections of their reservoir rodents that are clinically benign. Important variables such as the age of animals, route of infection, and the strain of virus are used to determine the outcome of infection and pathologic manifestations.

Natural infection with LCMV of newborn or fetal mice results in a chronic infection disseminated to many organs with high levels of viremia that apparently does not affect the growth and fertility of these animals and fails to induce an inflammatory response. However, peripheral infection of adult mice results in a transient immunizing infection. The use of inbred mice led to significant achievements in understanding of the mechanisms of T cell–mediated immunopathology, as pathogenicity of LCMV strains with experimentally manipulated genomes significantly differs in these animals. Although newborn mice develop chronic infection with viremia, T-cell immunity is suppressed. However, depending on the particular genotype of infected mice, different quantities of virus-specific antibodies are produced and form complexes with viral antigens. On one hand, trapping of circulating virus-antibody complexes in the glomerulus ultimately leads to the development of chronic glomerulonephritis.[67] On the other hand, intracerebral injection of adult mice produces acute fatal choriomeningitis that is characterized by extensive infiltration of cytotoxic T cells.[68]

The results of laboratory infections of natural reservoir *Calomys* rodents with JUNV or MACV resemble the outcome observed in LCMV-infected mice. However, the infection is never lethal in these animals by any route at any age, formation of antigen-antibody complexes have never been demonstrated, and some mice inoculated as adults develop chronic viremic infections, which completely mimic that observed in very young mice infected with LCMV. Also, chronic MACV infections can induce a microcytic hemolytic anemia that would result in chronic splenomegaly, which can be used as a marker for infection.[26,59,69]

Infection of *Mastomys* rodents with LASV similarly results in a chronic infection with no acute response and absence of inflammatory responses. Alternatively, some animals, depending on age, can mount an effective immune response and clear infection.[49] According to field studies, permanently infected animals have little or no specific antibodies but carry significant amounts of virus in tissues and body fluids, particularly in urine.[70]

Arenavirus infections of nonreservoir rodents result in benign, self-limited infection and immunity. Guinea pigs, however, may experience severe acute disease and serve as useful models of arenaviral hemorrhagic fever, in particular, AHF.[53]

Nonhuman Primates

Monkeys represent useful, although not optimal, models for studying pathogenesis and development of vaccines and therapeutic interventions to combat arenavirus infections in humans. LCMV readily infects macaque monkeys by inhalation of aerosolized viral particles, but infection results in a hemorrhagic fever rather than a central nervous system disease. Marmosets are also susceptible to lethal LCMV infection. Of note, feeding

of zoo monkeys with infected newborn mice caused sporadic fatal hepatic disease.[71] Several nonhuman primate species have been evaluated as potential models for LF, including squirrel monkeys, capuchin monkeys, marmosets, hamadryas baboons, African green monkeys, cynomolgus monkeys, and rhesus monkeys.[49,53,72–77] Experimental LASV infection of common marmosets results in systemic disease that has clinical and pathologic features highly similar to features observed in fatal cases of LF in human patients.[76] Small-particle aerosol infection induces a lethal disease in rhesus monkeys with pathologic findings similar to findings in humans.[25] MACV and JUNV are also pathogenic for nonhuman primates; however, the clinical and pathologic disease presentation is highly species specific. The use of nonhuman primates as well as guinea pig models that are more accessible for experimental work has led to significant advancements in our understanding of pathologic mechanisms during hemorrhagic fever diseases in humans.[53,78–80]

Humans

Limited histopathologic data are available from fatal human cases of LCM. However, a detailed description of a single case of human encephalitis shows a marked neuronal pattern of LCNV infection.[81]

The current knowledge of LF pathogenesis permits reliable re-creation of the chain of events that leads to disease development and fatal outcome. Considering the high mortality and dramatic course of the disease, the pathologic findings do not provide the basis that would explain the mechanism of disease progression. Very little or even absence of inflammatory response and variable levels of necrosis have been observed in liver, spleen, and adrenal glands of LASV-infected patients.[55,82] Although focal hepatic necrosis is commonly observed, the histopathologic changes in the liver are not sufficient to explain the lethal outcome. Data from experimentally infected animals suggest direct viral infection of endothelial cells and release of inflammatory mediators, possibly from infected macrophages, to be a central mechanism causing vascular dysfunction and, ultimately, shock in arenavirus hemorrhagic fevers.[82,83]

Patients who die of AHF and BHF also show very limited histopathologic changes in tissues. No vasculitis and virtually no inflammatory response is observed in the organs. However, small focal hemorrhages are commonly detected primarily in mucosal surfaces. On average, hepatic necrosis is more pronounced in cases of LF than AHF and BHF, but Councilman-like bodies are distinctly detectable in all three diseases. In addition, bronchopneumonia of either primary viral or secondary bacterial origin, which is more common, is often present. Concentrations of circulating endogenous interferon-α are highly predictive of the disease outcome in patients with AHF. The highest levels are reached 6 to 12 days after the onset of specific symptoms and indicate a poor prognosis for survival.[84] Moreover, experimental and clinical data suggest that interferons and high levels of proinflammatory cytokines may have a detrimental rather than beneficial effect in arenavirus infections.[85,86]

High titers of infectious virus and large amounts of viral antigens are detected in tissues of fatal cases of human infections and monkeys experimentally infected with LASV, JUNV, and MACV. Major replication sites of New World viruses JUNV and MACV are spleen, lymph nodes, and bone marrow, whereas LASV is found in many visceral organs and, notably, in placentas of pregnant women. Mesothelial surfaces are markedly infected with LASV and thus may further facilitate the development of serous effusions.[83]

CLINICAL MANIFESTATIONS

Clinically apparent infections with all the arenaviruses are similar in presenting manifestations. Fever is typically insidious in onset and is accompanied by headache and significant myalgia and malaise. Relative bradycardia is common, as is dysesthesia, particularly hyperesthesia of the skin. Thereafter the various diseases pursue different courses.

Lymphocytic Choriomeningitis Virus

LCMV infections are most commonly febrile illnesses with headache and systemic symptoms and are associated with leukopenia and thrombocytopenia.[26–31,87] After 3 to 5 days of nonspecific illness, occasionally with lymphadenopathy and a maculopapular rash, the fever subsides, but it frequently recurs in 2 to 4 days with several days of even more severe headache. Patients may exhibit frank meningitis during this second febrile period. Cerebrospinal fluid pressure usually is elevated, occasionally even with papilledema; the protein concentration ranges from 50 to 300 mg/dL; and several hundred lymphocytes per cubic millimeter are commonly observed. Hypoglycorrhachia is found in fewer than one-third of patients. Encephalomyelitic infection may manifest as encephalitis; psychosis; paraplegia; or disturbances of cranial, sensory, or autonomic nervous function. Ependymal inflammation has resulted in transient aqueductal stenosis.

Occasionally, patients develop orchitis, myocarditis, arthritis, or alopecia. Orchitis develops 1 to 3 weeks after the onset of the illness; it is usually unilateral and painful and resolves within 2 weeks. Myocarditis is detected by electrocardiographic changes and labile tachycardia during and after the second febrile period. Arthritis occurs occasionally during convalescence, principally affects the metacarpophalangeal and proximal interphalangeal joints, and is marked by minimal swelling and redness; it generally resolves within a few weeks.

The second febrile episode and some of the complications of convalescence have long been believed to represent immunopathologic phenomena. Antibodies detectable by immunofluorescence appear at about this time, and the lymphocytes in the cerebrospinal fluid presumably are analogues of the T lymphocytes that cause lymphocytic choriomeningitis in the intracerebrally inoculated adult mouse.

Additional data supporting this idea have gained support from unexpected findings in renal transplantation. Thirteen recipients of kidney, lung, or liver transplants from four organ donors developed severe disease with multiorgan involvement and clear evidence of LCMV infection.[88–90] None of the transplant recipients developed classic LCMV aseptic meningitis. All except one died with severe disease and disseminated infection similar to that seen in LF. This pattern has also been described in patients with tumors receiving LCMV as a supposed oncolytic therapy.[91] The only survivor had decreased immunosuppression and received intravenous ribavirin. Only one of the donors had documented contact with a rodent, a hamster shown to be infected with LCMV and coming from LCMV-infected pet suppliers.[92]

Lassa Fever

Most LASV infections result in either mild or subclinical disease manifestations and do not require hospitalization.[34] Severe hemorrhagic fever that involves multiple organs occurs in only 5% to 10% of cases of infection. Case-fatality rates among hospitalized patients consistently range from 15% to 25%. The incubation period of LF usually lasts 7 to 21 days.[32,54] The onset of clinical disease is characterized as a flulike illness with fever, general weakness, and malaise and may be accompanied by cough, sore throat, and severe headache. Common symptoms are nausea, vomiting, and diarrhea. The differential diagnosis of LF based on the presenting symptoms can be problematic due to the many other acute undifferentiated febrile illnesses circulating in West Africa.[32,44,54,93] A case-control study in patients hospitalized with LF was performed in Sierra Leone: 74% reported retrosternal chest pain; 60%, sore throat; 62%, back pain; 62%, cough; 50%, abdominal pain; 49%, vomiting; 26%, diarrhea; 25%, conjunctivitis; and 10%, facial edema. In addition, 43% of patients developed proteinuria. Mucosal bleeding at any time was observed in only 17% of LF cases. In this study, the combination of fever, pharyngitis, retrosternal pain, and proteinuria predicted 81% of laboratory-confirmed cases of LF. Fatal outcome was best predicted by the combination of fever, sore throat, and vomiting.[54] Involvement of the central nervous system is commonly characterized by encephalopathy, encephalitis, meningeal signs, and convalescent cerebral syndromes.[54,94]

The level of viremia is highly predictive of the disease outcome. In one study that involved 137 patients with LF, patients who developed viremia of $<10^3$ median tissue culture infectious dose ($TCID_{50}$)/mL on the day of hospitalization had 3.7 times greater chance of survival than patients admitted with higher virus loads in blood. Similarly, the probability of fatal outcome in patients with serum titers $>10^3$ $TCID_{50}$/mL and serum levels of aspartate aminotransferase (AST) ≥150 IU/L was 21 times higher than that in patients not meeting either of these criteria. In virtually all fatal cases, patients had high levels of viremia at the time of death, ranging from 10^3 to 10^8 $TCID_{50}$/mL.[95] Virus burden in blood reaches its maximum peak between day 4 and 9 after the onset of

symptomatic disease, which is followed by pronounced clinical manifestations. Recovering patients with LF clear virus from the bloodstream about 3 weeks after the beginning of illness.[39,95–97]

The clinical manifestations in fatal cases of LF generally occur during the second week of illness and include hypotension, peripheral vasoconstriction, reduced urinary output, facial and pulmonary edema, and occasionally pleural effusions and ascites. Minor hemorrhages are often observed in mucosal surfaces, which strongly suggests the involvement of diffuse capillary leakage. Moreover, these patients often experience myocardial depression that can potentially contribute to the increase in vascular permeability.[54] Patients who do not develop vascular leakage may have other complications during the second and third weeks of illness. Unilateral or bilateral eighth cranial nerve deafness is most commonly observed and was observed in almost one-third of hospitalized patients.[56] Some cases of sensorineural deafness are mild or improve with time; however, LF is a major cause of hearing loss in endemic areas.

South American Hemorrhagic Fevers

The South American hemorrhagic fevers resemble one another. The onset of the clinical disease is insidious, with anorexia, malaise, headache, chills, myalgias, and fever (38°C–39°C). A few days later the disease may progress and patients develop gastrointestinal, cardiovascular, and potentially neurologic signs and symptoms. Patients may also report retro-orbital pain, nausea or vomiting, epigastric pain, photophobia, dizziness, and constipation or mild diarrhea. Common absence of productive cough or nasal congestion is helpful to exclude initial symptoms of conditions caused by influenza or other acute respiratory infections.[98–100]

The first clinical manifestations of South American hemorrhagic fever are nonspecific, so the history of potential exposure to infected rodents is crucial. Among the infectious diseases, the differential diagnosis includes typhoid fever, hepatitis, infectious mononucleosis, leptospirosis, hantavirus pulmonary syndrome, dengue, dengue hemorrhagic fever, and rickettsioses. Malaria should also be considered in endemic areas. In the respective endemic areas or in patients with a history of travel to such areas, a febrile syndrome with proteinuria, leukopenia, and thrombocytopenia is suggestive of one of the South American hemorrhagic fevers.[101,102]

Intrauterine Infection

Arenaviruses readily invade the fetus, whether in their natural reservoir, in laboratory animals, or in humans. Pregnant women with LF often miscarry and have a high mortality rate,[103] and similar observations have been made in pregnant women with AHF and BHF. LCMV infection in pregnant women can lead to fetal infection, hydrocephalus, microcephaly, or chorioretinitis, or all of these disorders.[104–107] Because viral antibody rates indicate that about 5% of adults in large cities of the United States have been infected with LCMV, congenital infection may be more common than appreciated.[108,109]

DIAGNOSIS

The specific diagnosis depends on demonstration of the infecting virus or one of its products in acute serum samples. Reverse-transcriptase polymerase chain reaction is the most sensitive and fastest diagnostic method. In general, viremia and antigenemia are readily detected during the acute phase, when patients present with fever, and disappear as the patient recovers. The presence of viral nucleic acid can be detected during the same period and perhaps 1 or 2 days longer. Seroconversion, notably immunoglobulin M antibodies, may be detectable in hospitalized patients; however, antibodies usually appear early in convalescence. Diagnosis of initial patients in any outbreak benefits from study of virus isolates and classic serology. Most hemorrhagic fever viruses are hazardous and should be isolated or studied only under Biosafety Level 4 containment. Ideally, collected blood samples from patients with hemorrhagic fever should be collected early in the course of illness. Both serum and blood clot should be frozen as soon as possible and kept frozen until virology assays can be performed. A second serum sample must be collected before discharge for comparative serology. In fatal cases, a full autopsy should be performed, if possible, with a complete set of organs collected in formalin for diagnostic studies; spleen, liver, and lymph nodes should be collected frozen for virus isolation. Classic histopathology is often useful when yellow fever, Rift Valley fever, or a filovirus infection is suspected and when diagnosing some of the confounding diseases. Immunohistochemistry on fixed tissues can usually make a definitive diagnosis possible.

TREATMENT

Most information about treatment of hemorrhagic fevers in the Americas comes from reports from Argentina. AHF has a 6- to 14-day prodromic phase, and 8 to 12 days following its onset, about 20% to 30% of patients advance to the neurologic and hemorrhagic phase involving symptoms such as confusion, convulsions, coma, and bleeding from body orifices. Current recommended therapy of AHF involves treatment with immune plasma from convalescent patients that is very effective if initiated in the first week of disease.

Immune plasma therapy can ameliorate the immediate disease symptoms of AHF and significantly reduce mortality if administered early. However, in about 10% of cases, this therapy results in a late neurologic syndrome due to unknown mechanisms. Further limitations to the use of this treatment are dictated by a short supply of plasma. In vitro and in vivo studies have documented the prophylactic and therapeutic value of the nucleoside analogue ribavirin (Rib) against several arenaviruses including JUNV.[101,102,110,111] (Also see Chapter 45.) Importantly, Rib reduced both morbidity and mortality associated with JUNV infections in humans[101] when given early in the course of clinical disease. Some limitations of the use of Rib are its frequent and sometimes significant toxicities, including anemia and congenital disorders, and the need for intravenous administration for optimal efficacy.

The intravenous administration of Rib to patients with LF admitted to the hospital in Sierra Leone with AST elevations of at least 150 IU reduced the mortality rate from 55% to 5% when treatment was begun before day 7 of the disease.[112] However, a positive effect on survival was achieved at all stages of infection. Ribavirin has been used with apparent success in aborting a Sabia virus laboratory infection[113] and in treating patients with BHF.[114] Given the similarities to LASV and similar preclinical test efficacy, the drug should be considered for use in the treatment of any serious arenaviral infection. Close contacts of patients with arenaviral infections or possible bioterrorist exposures should not be given prophylactic ribavirin, but rather should be monitored for the appearance of fever.[115] Ribavirin therapy should be begun expectantly if fever is confirmed. Intravenous ribavirin is not licensed in the United States, and arenavirus treatment is not a US Food and Drug Administration–approved indication for the oral drug.

Supportive treatment is also highly recommended in hospitalized patients and consists of adequate hydration and effective management of the neurologic alterations, blood losses, shock, and additional superimposed infections, if acquired. In the case of AHF, pneumonia is the most common secondary bacterial infection. It can be accompanied by radiographic changes and an increase in fever, but not by leukocytosis; pneumonia usually responds to antibiotics. However, most of the severe reported clinical manifestations are neurologic, and it may be necessary to sedate agitated patients with diphenhydramine or diazepam; diazepam also gives some protection against seizures.

PREVENTION

Prevention of arenaviral infection may be approached by interdicting transmission from rodents to humans, from person to person, and from infected specimens to laboratory workers or by passive or active immunization. Community rodent control completely halted a major outbreak of BHF[116]; elimination of infected laboratory hamsters controlled LCMV outbreaks.[28,117] However, a household rodent control study of LASV infection yielded disappointing results.[118]

Person-to-person spread within hospitals has been a problem with LF. In endemic situations such as in Sierra Leone, partial spatial isolation of patients and the use of enteric precautions such as gloves, gowns, and careful disposal of patient wastes and fomites have generally served to prevent nosocomial outbreaks. Segregation of patients on the basis of the risk for death and likely content of virus in blood and body fluids by measuring AST levels should be of value. Rarely, hospital outbreaks appear to have been caused by infectious aerosols.[119,120] Thus wherever

practical, it is advisable to place patients in single rooms with isolated negative-pressure airflow and to provide medical staff with goggles and positive-pressure filtered air respirators or absolute filter respirators.[121] All potentially contaminated refuse or specimens should be double-bagged and the outer bag rinsed with 0.6% sodium hypochlorite before removal from the patient's room. Isolation of patients should continue until multiple blood and urine specimens are virus negative. Condoms should be used for a period in convalescence because of the occasional sexual transmission of arenaviral hemorrhagic fevers. In the recognized cases of LF that have been exported from Africa, no secondary transmission has yet been detected in contacts or in medical staff, indicating that a conservative approach to isolation provides a reasonable standard of safe care in most cases.

Laboratory-acquired infection is a major problem because all arenaviruses are infectious as aerosols. Several infections, some fatal, have occurred in laboratory workers. Thus it is imperative that work with all arenaviruses except LCMV be conducted in special laboratories with Biosafety Level 4 containment. Clinical pathologic tests, particularly in LF patients, also present problems. Aerosols must be minimized or contained. Acid treatment inactivates virus for leukocyte counts, and alcohol fixation is useful for blood smears; heating serum at 60°C for 1 hour is feasible for measuring heat-stable substances.[122]

There are no arenavirus vaccines licensed in the United States. Human vaccine against AHF is available in Argentina. The vaccine was produced by serial passages prototype XJ strain of JUNV in newborn mice. This resulted in the generation of Candid 1 vaccine, which was generated from the 44th mouse brain passage of XJ strain of JUNV and found to be attenuated in guinea pigs. Preclinical studies at the US Army Medical Research Institute of Infectious Diseases supported the safety, immunogenicity, and protective efficacy of Candid 1 in both guinea pigs and rhesus macaques. Subsequent clinical studies performed in Argentina have shown Candid 1 to be an effective and safe vaccine in humans.[123] This vaccine received approval for human use in 2006 by the regulatory agency of Argentina for use exclusively in Argentina. Experimental data from animal models indicate cross-protection of this vaccine against Machupo virus.

Key References

The complete reference list is available online at Expert Consult.

1. Murphy FA, Whitfield SG. Morphology and morphogenesis of arenaviruses. *Bull World Health Organ*. 1975;52:409–419.
2. Buchmeier MJ, Peters CJ, de la Torre JC. In: Knipe DM, Holey PM, eds. *Fields Virology*. Vol. 2. *Fifth ed*. 2007:1792–1827.
3. Franze-Fernandez M-T, et al. Molecular structure and early events in the replication of Tacaribe arenavirus S RNA. *Virus Res*. 1987;7:309–324.
4. Meyer BJ, Southern PJ. Concurrent sequence analysis of 5' and 3' RNA termini by intramolecular circularization reveals 5' nontemplated bases and 3' terminal heterogeneity for lymphocytic choriomeningitis virus mRNAs. *J Virol*. 1993;67:2621–2627.
5. Qi X, et al. Cap binding and immune evasion revealed by Lassa nucleoprotein structure. *Nature*. 2010;468:779–783.
6. Lelke M, Brunotte L, Busch C, et al. An N-terminal region of Lassa virus L protein plays a critical role in transcription but not replication of the virus genome. *J Virol*. 2010;84:1934–1944.
7. Morin B, et al. The N-terminal domain of the arenavirus L protein is an RNA endonuclease essential in mRNA transcription. *PLoS Pathog*. 2010;6:e1001038.
8. Cornu TI, de la Torre JC. RING finger Z protein of lymphocytic choriomeningitis virus (LCMV) inhibits transcription and RNA replication of an LCMV S-segment minigenome. *J Virol*. 2001;75:9415–9426.
9. Cornu TI, de la Torre JC. Characterization of the arenavirus RING finger Z protein regions required for Z-mediated inhibition of viral RNA synthesis. *J Virol*. 2002;76:6678–6688.
10. Kranzusch PJ, Whelan SPJ. Arenavirus Z protein controls viral RNA synthesis by locking a polymerase–promoter complex. *Proc Natl Acad Sci USA*. 2011;108:19743–19748.
11. Lopez N, Jacamo R, Franze-Fernandez MT. Transcription and RNA replication of tacaribe virus genome and antigenome analogs require N and L proteins: Z protein is an inhibitor of these processes. *J Virol*. 2001;75:12241–12251.
12. Hass M, Golnitz U, Muller S, et al. Replicon system for Lassa virus. *J Virol*. 2004;78:13793–13803.
13. Rojek JM, Kunz S. Cell entry by human pathogenic arenaviruses. *Cell Microbiol*. 2008;10:828–835.
14. Flanagan ML, et al. New World clade B arenaviruses can use transferrin receptor 1 (TfR1)-dependent and -independent entry pathways, and glycoproteins from human pathogenic strains are associated with the use of TfR1. *J Virol*. 2008;82:938–948.
15. Martinez-Sobrido L, Zuniga EI, Rosario D, et al. Inhibition of the type I interferon response by the nucleoprotein of the prototypic arenavirus lymphocytic choriomeningitis virus. *J Virol*. 2006;80:9192–9199.
16. Martinez-Sobrido L, Giannakas P, Cubitt B, et al. Differential inhibition of type I interferon induction by arenavirus nucleoproteins. *J Virol*. 2007;81:12696–12703.
17. Martínez-Sobrido L, et al. Identification of amino acid residues critical for the anti-interferon activity of the nucleoprotein of the prototypic arenavirus lymphocytic choriomeningitis virus. *J Virol*. 2009;83:11330–11340.
18. Eckerle LD, et al. Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. *PLoS Pathog*. 2010;6:e1000896.
19. Campbell Dwyer EJ, Lai H, MacDonald RC, et al. The lymphocytic choriomeningitis virus RING protein Z associates with eukaryotic initiation factor 4E and selectively represses translation in a RING-dependent manner. *J Virol*. 2000;74:3293–3300.
20. Borden KLB, Campbell Dwyer EJ, Carlile GW, et al. Two RING finger proteins, the oncoprotein PML and the arenavirus Z protein, colocalize with the nuclear fraction of the ribosomal P proteins. *J Virol*. 1998;72:3819–3826.
21. Fan L, Briese T, Lipkin WI. Z proteins of New World arenaviruses bind RIG-I and interfere with type I interferon induction. *J Virol*. 2010;84:1785–1791.
22. Childs JE, Glass GE, Korch GW, et al. Lymphocytic choriomeningitis virus infection and house mouse (*Mus musculus*) distribution in urban Baltimore. *Am J Trop Med Hyg*. 1992;47:27–34.
23. Armstrong C. Studies on choriomeningitis and poliomyelitis: Harvey Lecture, October 31, 1940. *Bull N Y Acad Med*. 1941;17:295–318.
24. Ackermann R. *Epidemiological Aspects of Lymphocytic Choriomeningitis in Man*. Springer-Verlag; 1973:234–237.
25. Stephenson EH, Larson EW, Dominik JW. Effect of environmental factors on aerosol-induced lassa virus infection. *J Med Virol*. 1984;14:295–303.
26. Enria DA, Mills JN, Bausch DG, et al. In: Guerrant RL, Walker DH, Weller PF, eds. *Tropical Infectious Diseases: Principles, Pathogenesis, Practice*. Ch. 68. Elsevier Saunders; 2011:449–461.
27. Hinman AR, et al. Outbreak of lymphocytic choriomeningitis virus infections in medical center personnel. *Am J Epidemiol*. 1975;101:103–110.
28. Baum SG, Lewis AM, Rowe WP, et al. Epidemic nonmeningitic lymphocytic-choriomeningitis-virus infection. *N Engl J Med*. 1966;274:934–936.
29. Dykewicz CA, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. *JAMA*. 1992;267:1349–1353.
30. Biggar RJ, Woodall JP, Walter PD, et al. Lymphocytic choriomeningitis outbreak associated with pet hamsters: Fifty-seven cases from New York State. *JAMA*. 1975;232:494–500.
31. Ackermann R, et al. Syrische Goldhamster als Uberträger von Lymphozytärer Choriomeningitis. *Dtsch Med Wochenschr*. 1972;97:1725–1731.
32. Frame JD, Baldwin JM, Gocke DJ, et al. Lassa fever, a new virus disease of man from West Africa. *Am J Trop Med Hyg*. 1970;19:670–676.
33. Buckley SM, Casals J. Lassa fever, a new virus disease of man from West Africa. 3. Isolation and characterization of the virus. *Am J Trop Med Hyg*. 1970;19:680–691.
34. McCormick JB, Webb PA, Krebs JW, et al. A prospective study of the epidemiology and ecology of Lassa fever. *J Infect Dis*. 1987;155:437–444.
35. Monath TP, Maher M, Casals J, et al. Lassa fever in the Eastern Province of Sierra Leone, 1970-1972. II. Clinical observations and virological studies on selected hospital cases. *Am J Trop Med Hyg*. 1974;23:1140–1149.
36. Lukashevich IS, Clegg JCS, Sidibe K. Lassa virus activity in Guinea: distribution of human antiviral antibody defined using enzyme-linked immunosorbent assay with recombinant antigen. *J Med Virol*. 1993;40:210–217.
37. Meulen JT, et al. Hunting of peridomestic rodents and consumption of their meat as possible risk factors for rodent-to-human transmission of Lassa virus in the Republic of Guinea. *Am J Trop Med Hyg*. 1996;55:661–666.
38. Frame JD, Yalley-Ogunro JE, Hanson AP. Endemic Lassa fever in Liberia. V. Distribution of Lassa virus activity in Liberia: hospital staff surveys. *Trans R Soc Trop Med Hyg*. 1984;78:761–763.
39. Monath TP, et al. A hospital epidemic of Lassa fever in Zorzor, Liberia, March-April 1972. *Am J Trop Med Hyg*. 1973;22:773–779.
40. Monson MH, Frame JD, Jahrling PB, et al. Endemic Lassa fever in Liberia. I. Clinical and epidemiological aspects at Curran Lutheran Hospital, Zorzor, Liberia. *Trans R Soc Trop Med Hyg*. 1984;78:549–553.
41. Carey DE, et al. Lassa fever epidemiological aspects of the 1970 epidemic, Jos, Nigeria. *Trans R Soc Trop Med Hyg*. 1972;66:402–408.
42. Tomori O, Fabiyi A, Sorungbe A, et al. Viral hemorrhagic fever antibodies in Nigerian populations. *Am J Trop Med Hyg*. 1988;38:407–410.
43. White HA. Lassa fever. A study of 23 hospital cases. *Trans R Soc Trop Med Hyg*. 1972;66:390–401.
44. Frame JD. Surveillance of Lassa fever in missionaries stationed in West Africa. *Bull World Health Organ*. 1975;52:593–598.
45. Günther S, et al. Imported Lassa fever in Germany: molecular characterization of a new Lassa virus strain. *Emerg Infect Dis*. 2000;6:466–476.
46. Gonzalez JP, Emonet S, Lamballerie XD, et al. In: Childs James E., Mackenzie John S., Richt Jürgen A., eds. *Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and Consequences of Cross-Species Transmission*. Vol. 315. Current Topics in Microbiology and Immunology. Springer Berlin Heidelberg; 2007:253–288.
47. Safronetz D, et al. Detection of Lassa virus, Mali. *Emerg Infect Dis*. 2010;16:1123–1126.
48. Monath TP, Newhouse VF, Kemp GE, et al. Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone. *Science*. 1974;185:263–265.
49. Walker DH, Wulff H, Lange JV, et al. Comparative pathology of Lassa virus infection in monkeys, guinea pigs, and *Mastomys natalensis*. *Bull World Health Organ*. 1975;52:523–535.
50. Salazar-Bravo J, Ruedas LA, Yates TL. Mammalian reservoirs of arenaviruses. *Curr Top Microbiol Immunol*. 2002;262:25–63.
51. Centers for Disease Control and Prevention. Lassa Fever Fact Sheet; 2012. http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/Fact_Sheets/Lassa_Fever_Fact_Sheet.pdf.
52. Keenlyside RA, et al. Case-control study of *Mastomys natalensis* and humans in Lassa virus-infected households in Sierra Leone. *Am J Trop Med Hyg*. 1983;32:829–837.
53. Peters CJ, et al. Experimental studies of arenaviral hemorrhagic fevers. *Curr Top Microbiol Immunol*. 1987;134:5–68.
54. McCormick JB, et al. A case-control study of the clinical diagnosis and course of Lassa fever. *J Infect Dis*. 1987;155:445–455.
55. Walker DH, et al. Pathologic and virologic study of fatal Lassa fever in man. *Am J Pathol*. 1982;107:349–356.
56. Cummins D, et al. Acute sensorineural deafness in Lassa fever. *JAMA*. 1990;264:2093–2096.
57. Briese T, et al. Genetic detection and characterization of Lujo virus, a new hemorrhagic fever–associated arenavirus from southern Africa. *PLoS Pathog*. 2009;5:e1000455.

58. Coimbra TL, et al. New arenavirus isolated in Brazil. *Lancet*. 1994;343:391–392.
59. Sabattini MS, Maiztegui JI. Fiebre hemorragica Argentina. *Medicina (B Aires)*. 1970;30:111–128.
60. Buchmeier MJ, de la Torre J, Peters CJ. In: Howley PM, Knipe DM, eds. *Fields Virology*. Wolter Kluwer Lippincott Williams, Wilkins. 2007:1791–1827.
61. Enria DA, Barrera Oro JG. Junin virus vaccines. *Curr Top Microbiol Immunol*. 2002;263:239–261.
62. Peters CJ, et al. Hemorrhagic fever in Cochabamba, Bolivia. *Am J Epidemiol*. 1971;99:425–433 (1974.
63. Kilgore PE, et al. Prospects for the control of Bolivian hemorrhagic fever. *Emerg Infect Dis*. 1995;1:97–100.
64. Mills JN, et al. A longitudinal study of Junin virus activity in the rodent reservoir of Argentine hemorrhagic fever. *Am J Trop Med Hyg*. 1992;47:749–763.
65. Salas R, et al. Venezuelan haemorrhagic fever. *Lancet*. 1991;338:1033–1036.
66. Milazzo ML, Campbell GL. Fulhorst, C. F. Novel arenavirus infection in humans, United States. *Emerg Infect Dis*. 2011;17:1417–1420.
67. Oldstone MBA, Dixon FJ. Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection: II. Relationship of the anti-lymphocytic choriomeningitis immune response to tissue injury in chronic lymphocytic choriomeningitis disease. *J Exp Med*. 1970;131:1–19.
68. Nathanson N, et al. *Virus-Induced Cell-Mediated Immunopathological Disease*. Academic Press; 1975.
69. Johnson KM, Kuns ML, Mackenzie RB, et al. Isolation of Machupo virus from wild rodent *Calomys callosus*. *Am J Trop Med Hyg*. 1966;15:103–106.
70. Demby AH, et al. Lassa fever in Guinea: II. Distribution and prevalence of Lassa virus infection in small mammals. *Vector Borne Zoonotic Dis*. 2001;1:283–297.
71. Montali RJ, et al. A common-source outbreak of callitrichid hepatitis in captive tamarins and marmosets. *J Infect Dis*. 1993;167:946–950.
72. Walker DH. Pathology and pathogenesis of arenavirus infections. *Curr Top Microbiol Immunol*. 1987;133:89–113.
73. Walker DH, Wulff H, Murphy FA. Experimental Lassa virus infection in the squirrel monkey. *Am J Pathol*. 1975;80:261–278.
74. Walker DH, et al. Experimental infection of Rhesus monkeys with Lassa virus and a closely related arenavirus, Mozambique virus. *J Infect Dis*. 1982;146:360–368.
75. Evseev AA, Dvoretskaia VI, Bogatikov GV, et al. Experimental Lassa fever in hamadryas baboons. *Vopr Virusol*. 1991;36:150–152.
76. Carrion R Jr, et al Vol. 81 6482-6490 (2007).
77. Hensley L, et al. Pathogenesis of lassa fever in cynomolgus macaques. *Virol J*. 2011;8:205.
78. Yun NE, et al. Pathogenesis of XJ and Romero strains of Junin virus in two strains of guinea pigs. *Am J Trop Med Hyg*. 2008;79:275–282.
79. Emonet SF, et al. Rescue from cloned cDNAs and in vivo characterization of recombinant pathogenic romero and life-attenuated Candid #1 strains of Junin virus, the causative agent of Argentine hemorrhagic fever disease. *J Virol*. 2010;85:1473–1483.
80. McKee KT Jr, Mahlandt BG, Maiztegui JI, et al. Experimental Argentine hemorrhagic fever in rhesus macaques: viral strain-dependent clinical response. *J Infect Dis*. 1985;152:218–221.
81. Warkel RL, et al. Fatal acute meningoencephalitis due to lymphocytic choriomeningitis virus. *Neurology*. 1973;23: 198–203.
82. Yun NE, Walker DH. Pathogenesis of Lassa fever. *Viruses*. 2012;4:2031–2048.
83. Zaki SR, Peters CJ. In: Connor DH, et al, eds. *The Pathology of Infectious Diseases*. Appleton, Lange; 1997:347–364.
84. Levis SC, et al. Correlation between endogenous interferon and the clinical evolution of patients with Argentine hemorrhagic fever. *J Interferon Res*. 1985;5:383–389.
85. Vilcek J. Adverse effects of interferon in virus infections, autoimmune diseases and acquired immunodeficiency. *Prog Med Virol*. 1984;30:62–77.
86. Marta RF, et al. Proinflammatory cytokines and elastase-alpha-1-antitrypsin in Argentine hemorrhagic fever. *Am J Trop Med Hyg*. 1999;60:85–89.
87. Dykewicz CA, Dato VM, Fisher-Hoch SP, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. *JAMA*. 1992;267:1349–1353.
93. Schmitz H, et al. Monitoring of clinical and laboratory data in two cases of imported Lassa fever. *Microbes Infect*. 2002;4:43–50.
94. Solbrig MV. *Lassa Virus and Central Nervous System Diseases*. Plenum; 1993:325–330.
95. Johnson KM, et al. Clinical virology of Lassa fever in hospitalized patients. *J Infect Dis*. 1987;155:456–464.
96. Demby AH, Chamberlain J, Brown DW, et al. Early diagnosis of Lassa fever by reverse transcription-PCR. *J Clin Microbiol*. 1994;32:2898–2903.
97. Trappier SG, et al. Evaluation of the polymerase chain reaction for diagnosis of Lassa virus infection. *Am J Trop Med Hyg*. 1993;49:214–221.
98. Douglas RG, Wiebenga NH, Couch RB. Bolivian hemorrhagic fever probably transmitted by personal contact. *Am J Epidemiol*. 1965;82:85–91.
99. Maiztegui JI. Clinical and epidemiological patterns of Argentine haemorrhagic fever. *Bull World Health Organ*. 1975;52:567–575.
100. Stinebaugh BJ, et al. Bolivian hemorrhagic fever: a report of four cases. *Am J Med*. 1966;40:217–230.
101. McKee KT Jr, Huggins JW, Trahan CJ, et al. Ribavirin prophylaxis and therapy for experimental Argentine hemorrhagic fever. *Antimicrob Agents Chemother*. 1988;32:1304–1309.
102. Andrei G, De Clercq E. Inhibitory effect of selected antiviral compounds on arenavirus replication in vitro. *Antiviral Res*. 1990;14:287–299.
109. Briggiler AM, Enria DA, Maiztegui JI. Argentine hemorrhagic fever in pregnant women. *Medicina (B Aires)*. 1990;50.
110. Damonte EB, Coto CE. Treatment of arenavirus infections: from basic studies to the challenge of antiviral therapy. *Adv Virus Res*. 2002;58:125–155.
111. Andrei G, De Clercq E. Molecular approaches for the treatment of hemorrhagic fever virus infections. *Antiviral Res*. 1993;22:45–75.
123. Maiztegui JI, et al. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. *J Infect Dis*. 1998;177:277–283.

References

1. Murphy FA, Whitfield SG. Morphology and morphogenesis of arenaviruses. *Bull World Health Organ*. 1975;52:409–419.
2. Buchmeier MJ, Peters CJ, de la Torre JC in Fields Virology, Fifth Edition Vol. 2 (ed D. M. Knipe, Holey, P.M.) 1792-1827 (2007).
3. Franze-Fernandez M-T, et al. Molecular structure and early events in the replication of Tacaribe arenavirus S RNA. *Virus Res*. 1987;7:309–324.
4. Meyer BJ, Southern PJ. Concurrent sequence analysis of 5' and 3' RNA termini by intramolecular circularization reveals 5' nontemplated bases and 3' terminal heterogeneity for lymphocytic choriomeningitis virus mRNAs. *J Virol*. 1993;67:2621–2627.
5. Qi X, et al. Cap binding and immune evasion revealed by Lassa nucleoprotein structure. *Nature*. 2010;468:779–783.
6. Lelke M, Brunotte L, Busch C, et al. An N-terminal region of Lassa virus L protein plays a critical role in transcription but not replication of the virus genome. *J Virol*. 2010;84:1934–1944.
7. Morin B, et al. The N-terminal domain of the arenavirus L protein is an RNA endonuclease essential in mRNA transcription. *PLoS Pathog*. 2010;6:e1001038.
8. Cornu TI, de la Torre JC. RING finger Z protein of lymphocytic choriomeningitis virus (LCMV) inhibits transcription and RNA replication of an LCMV S-segment minigenome. *J Virol*. 2001;75:9415–9426.
9. Cornu TI, de la Torre JC. Characterization of the arenavirus RING finger Z protein regions required for Z-mediated inhibition of viral RNA synthesis. *J Virol*. 2002;76:6678–6688.
10. Kranzusch PJ, Whelan SPJ. Arenavirus Z protein controls viral RNA synthesis by locking a polymerase–promoter complex. *Proc Natl Acad Sci USA*. 2011;108:19743–19748.
11. Lopez N, Jacamo R, Franze-Fernandez MT. Transcription and RNA replication of tacaribe virus genome and antigenome analogs require N and L proteins: z protein is an inhibitor of these processes. *J Virol*. 2001;75:12241–12251.
12. Hass M, Golnitz U, Muller S, et al. Replicon system for Lassa virus. *J Virol*. 2004;78:13793–13803.
13. Rojek JM, Kunz S. Cell entry by human pathogenic arenaviruses. *Cell Microbiol*. 2008;10:828–835.
14. Flanagan ML, et al. New World clade B arenaviruses can use transferrin receptor 1 (TfR1)-dependent and -independent entry pathways, and glycoproteins from human pathogenic strains are associated with the use of TfR1. *J Virol*. 2008;82:938–948.
15. Martinez-Sobrido L, Zuniga EI, Rosario D, et al. Inhibition of the type I interferon response by the nucleoprotein of the prototypic arenavirus lymphocytic choriomeningitis virus. *J Virol*. 2006;80:9192–9199.
16. Martinez-Sobrido L, Giannakas P, Cubitt B, et al. Differential inhibition of type I interferon induction by arenavirus nucleoproteins. *J Virol*. 2007;81:12696–12703.
17. Martínez-Sobrido L, et al. Identification of amino acid residues critical for the anti-interferon activity of the nucleoprotein of the prototypic arenavirus lymphocytic choriomeningitis virus. *J Virol*. 2009;83:11330–11340.
18. Eckerle LD, et al. Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. *PLoS Pathog*. 2010;6:e1000896.
19. Campbell Dwyer EJ, Lai H, MacDonald RC, et al. The lymphocytic choriomeningitis virus RING protein Z associates with eukaryotic initiation factor 4E and selectively represses translation in a RING-dependent manner. *J Virol*. 2000;74:3293–3300.
20. Borden KLB, Campbell Dwyer EJ, Carlile GW, et al. Two RING finger proteins, the oncoprotein PML and the arenavirus Z protein, colocalize with the nuclear fraction of the ribosomal P proteins. *J Virol*. 1998;72:3819–3826.
21. Fan L, Briese T, Lipkin WI. Z proteins of New World arenaviruses bind RIG-I and interfere with type I interferon induction. *J Virol*. 2010;84:1785–1791.
22. Childs JE, Glass GE, Korch GW, et al. Lymphocytic choriomeningitis virus infection and house mouse (*Mus musculus*) distribution in urban Baltimore. *Am J Trop Med Hyg*. 1992;47:27–34.
23. Armstrong C. Studies on choriomeningitis and poliomyelitis: Harvey Lecture, October 31, 1940. *Bull N Y Acad Med*. 1941;17:295–318.
24. Ackermann R. *Epidemiological Aspects of Lymphocytic Choriomeningitis in Man*. Springer-Verlag; 1973:234–237.
25. Stephenson EH, Larson EW, Dominik JW. Effect of environmental factors on aerosol-induced Lassa virus infection. *J Med Virol*. 1984;14:295–303.
26. Enria DA, Mills JN, Bausch DG, et al. In: Guerrant RL, Walker DH, Weller PF, eds. *Tropical Infectious Diseases: Principles, Pathogenesis, Practice*. Ch. 68. Elsevier Saunders; 2011:449–461.
27. Hinman AR, et al. Outbreak of lymphocytic choriomeningitis virus infections in medical center personnel. *Am J Epidemiol*. 1975;101:103–110.
28. Baum SG, Lewis AM, Rowe WP, et al. Epidemic nonmeningitic lymphocytic-choriomeningitis-virus infection. *N Engl J Med*. 1966;274:934–936.
29. Dykewicz CA, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. *JAMA*. 1992;267:1349–1353.
30. Biggar RJ, Woodall JP, Walter PD, et al. Lymphocytic choriomeningitis outbreak associated with pet hamsters: Fifty-seven cases from New York State. *JAMA*. 1975;232:494–500.
31. Ackermann R, et al. Syrische Goldhamster als Uberträger von Lymphozytärer Choriomeningitis. *Dtsch Med Wochenschr*. 1972;97:1725–1731.
32. Frame JD, Baldwin JM, Gocke DJ, et al. Lassa fever, a new virus disease of man from West Africa. *Am J Trop Med Hyg*. 1970;19:670–676.
33. Buckley SM, Casals J. Lassa fever, a new virus disease of man from West Africa. 3. Isolation and characterization of the virus. *Am J Trop Med Hyg*. 1970;19:680–691.
34. McCormick JB, Webb PA, Krebs JW, et al. A prospective study of the epidemiology and ecology of Lassa fever. *J Infect Dis*. 1987;155:437–444.
35. Monath TP, Maher M, Casals J, et al. Lassa fever in the Eastern Province of Sierra Leone, 1970-1972. II. Clinical observations and virological studies on selected hospital cases. *Am J Trop Med Hyg*. 1974;23:1140–1149.
36. Lukashevich IS, Clegg JCS, Sidibe K. Lassa virus activity in Guinea: distribution of human antiviral antibody defined using enzyme-linked immunosorbent assay with recombinant antigen. *J Med Virol*. 1993;40:210–217.
37. Meulen JT, et al. Hunting of peridomestic rodents and consumption of their meat as possible risk factors for rodent-to-human transmission of Lassa virus in the Republic of Guinea. *Am J Trop Med Hyg*. 1996;55:661–666.
38. Frame JD, Yalley-Ogunro JE, Hanson AP. Endemic Lassa fever in Liberia. V. Distribution of Lassa virus activity in Liberia: hospital staff surveys. *Trans R Soc Trop Med Hyg*. 1984;78:761–763.
39. Monath TP, et al. A hospital epidemic of Lassa fever in Zorzor, Liberia, March-April 1972. *Am J Trop Med Hyg*. 1973;22:773–779.
40. Monson MH, Frame JD, Jahrling PB, et al. Endemic Lassa fever in Liberia. I. Clinical and epidemiological aspects at Curran Lutheran Hospital, Zorzor, Liberia. *Trans R Soc Trop Med Hyg*. 1984;78:549–553.
41. Carey DE, et al. Lassa fever epidemiological aspects of the 1970 epidemic, Jos, Nigeria. *Trans R Soc Trop Med Hyg*. 1972;66:402–408.
42. Tomori O, Fabiyi A, Sorungbe A, et al. Viral hemorrhagic fever antibodies in Nigerian populations. *Am J Trop Med Hyg*. 1988;38:407–410.
43. White HA. Lassa fever. A study of 23 hospital cases. *Trans R Soc Trop Med Hyg*. 1972;66:390–401.
44. Frame JD. Surveillance of Lassa fever in missionaries stationed in West Africa. *Bull World Health Organ*. 1975;52:593–598.
45. Günther S, et al. Imported Lassa fever in Germany: molecular characterization of a new Lassa virus strain. *Emerg Infect Dis*. 2000;6:466–476.
46. Gonzalez JP, Emonet S, Lamballerie X. d., et al. In: Childs James E., Mackenzie John S., Richt Jürgen A., eds. *Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and Consequences of Cross-Species Transmission*. Vol. 315. Current Topics in Microbiology and Immunology. Springer Berlin Heidelberg; 2007:253–288.
47. Safronetz D, et al. Detection of Lassa virus, Mali. *Emerg Infect Dis*. 2010;16:1123–1126.
48. Monath TP, Newhouse VF, Kemp GE, et al. Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone. *Science*. 1974;185:263–265.
49. Walker DH, Wulff H, Lange JV, et al. Comparative pathology of Lassa virus infection in monkeys, guinea pigs, and *Mastomys natalensis*. *Bull World Health Organ*. 1975;52:523–535.
50. Salazar-Bravo J, Ruedas LA, Yates TL. Mammalian reservoirs of arenaviruses. *Curr Top Microbiol Immunol*. 2002;262:25–63.
51. Centers for Disease Control and Prevention. Lassa Fever Fact Sheet; 2012. http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/Fact_Sheets/Lassa_Fever_Fact_Sheet.pdf.
52. Keenlyside RA, et al. Case-control study of *Mastomys natalensis* and humans in Lassa virus-infected households in Sierra Leone. *Am J Trop Med Hyg*. 1983;32:829–837.
53. Peters CJ, et al. Experimental studies of arenaviral hemorrhagic fevers. *Curr Top Microbiol Immunol*. 1987;134:5–68.
54. McCormick JB, et al. A case-control study of the clinical diagnosis and course of Lassa fever. *J Infect Dis*. 1987;155:445–455.
55. Walker DH, et al. Pathologic and virologic study of fatal Lassa fever in man. *Am J Pathol*. 1982;107:349–356.
56. Cummins D, et al. Acute sensorineural deafness in Lassa fever. *JAMA*. 1990;264:2093–2096.
57. Briese T, et al. Genetic detection and characterization of Lujo virus, a new hemorrhagic fever–associated arenavirus from southern Africa. *PLoS Pathog*. 2009;5:e1000455.
58. Coimbra TL, et al. New arenavirus isolated in Brazil. *Lancet*. 1994;343:391–392.
59. Sabattini MS, Maiztegui JI. Fiebre hemorragica Argentina. *Medicina (B Aires)*. 1970;30:111–128.
60. Buchmeier MJ, de la Torre J, Peters CJ. In: Howley PM, Knipe DM, eds. *Fields Virology*. Wolter Kluwer Lippincott Williams, Wilkins. 2007:1791–1827.
61. Enria DA, Barrera Oro JG. Junin virus vaccines. *Curr Top Microbiol Immunol*. 2002;263:239–261.
62. Peters CJ, et al. Hemorrhagic fever in Cochabamba, Bolivia. *Am J Epidemiol*. 1971;99:425–433 (1974.
63. Kilgore PE, et al. Prospects for the control of Bolivian hemorrhagic fever. *Emerg Infect Dis*. 1995;1:97–100.
64. Mills JN, et al. A longitudinal study of Junin virus activity in the rodent reservoir of Argentine hemorrhagic fever. *Am J Trop Med Hyg*. 1992;47:749–763.
65. Salas R, et al. Venezuelan haemorrhagic fever. *Lancet*. 1991;338:1033–1036.
66. Milazzo ML, Campbell GL. Fulhorst, C. F. Novel arenavirus infection in humans, United States. *Emerg Infect Dis*. 2011;17:1417–1420.
67. Oldstone MBA, Dixon FJ. Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection: II. Relationship of the anti-lymphocytic choriomeningitis immune response to tissue injury in chronic lymphocytic choriomeningitis disease. *J Exp Med*. 1970;131:1–19.
68. Nathanson N, et al. *Virus-induced cell-mediated immunopathological disease*. Academic Press; 1975.
69. Johnson KM, Kuns ML, Mackenzie RB, et al. Isolation of Machupo virus from wild rodent *Calomys callosus*. *Am J Trop Med Hyg*. 1966;15:103–106.
70. Demby AH, et al. Lassa fever in Guinea: II. Distribution and prevalence of Lassa virus infection in small mammals. *Vector Borne Zoonotic Dis*. 2001;1:283–297.71. Montali RJ, et al. A common-source outbreak of callitrichid hepatitis in captive tamarins and marmosets. *J Infect Dis*. 1993;167:946–950.
72. Walker DH. Pathology and pathogenesis of arenavirus infections. *Curr Top Microbiol Immunol*. 1987;133:89–113.
73. Walker DH, Wulff H, Murphy FA. Experimental Lassa virus infection in the squirrel monkey. *Am J Pathol*. 1975;80:261–278.
74. Walker DH, et al. Experimental infection of Rhesus monkeys with Lassa virus and a closely related arenavirus, Mozambique virus. *J Infect Dis*. 1982;146:360–368.
75. Evseev AA, Dvoretskaia VI, Bogatikov GV, et al. Experimental Lassa fever in hamadryas baboons. *Vopr Virusol*. 1991;36:150–152.
76. Carrion R Jr, et al Vol. 81 6482-6490 (2007).
77. Hensley L, et al. Pathogenesis of lassa fever in cynomolgus macaques. *Virol J*. 2011;8:205.
78. Yun NE, et al. Pathogenesis of XJ and Romero strains of Junin virus in two strains of guinea pigs. *Am J Trop Med Hyg*. 2008;79:275–282.
79. Emonet SF, et al. Rescue from cloned cDNAs and in vivo characterization of recombinant pathogenic Romero and life-attenuated Candid #1 strains of Junin virus, the causative agent of Argentine hemorrhagic fever disease. *J Virol*. 2010;85:1473–1483.
80. McKee KT Jr, Mahlandt BG, Maiztegui JI, et al. Experimental Argentine hemorrhagic fever in rhesus macaques: viral strain-dependent clinical response. *J Infect Dis*. 1985;152:218–221.
81. Warkel RL, et al. Fatal acute meningoencephalitis due to lymphocytic choriomeningitis virus. *Neurology*. 1973;23:198–203.
82. Yun NE, Walker DH. Pathogenesis of Lassa fever. *Viruses*. 2012;4:2031–2048.
83. Zaki SR, Peters CJ. In: Connor DH, et al, eds. *The Pathology of Infectious Diseases*. Appleton, Lange; 1997:347–364.
84. Levis SC, et al. Correlation between endogenous interferon and the clinical evolution of patients with Argentine hemorrhagic fever. *J Interferon Res*. 1985;5:383–389.
85. Vilcek J. Adverse effects of interferon in virus infections, autoimmune diseases and acquired immunodeficiency. *Prog Med Virol*. 1984;30:62–77.

86. Marta RF, et al. Proinflammatory cytokines and elastase-alpha-1-antitrypsin in Argentine hemorrhagic fever. *Am J Trop Med Hyg*. 1999;60:85–89.
87. Dykewicz CA, Dato VM, Fisher-Hoch SP, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. *JAMA*. 1992;267:1349–1353.
88. Fischer SA, Graham MB, Kuehnert MJ, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation. *N Engl J Med*. 2006;354:2235–2249.
89. Palacios G, Druce J, Du L, et al. A new arenavirus in a cluster of fatal transplant-associated diseases. *N Engl J Med*. 2008;358:991–998.
90. Centers for Disease Control and Prevention. Brief report: lymphocytic choriomeningitis virus transmitted through solid organ transplantation—Massachusetts, 2008. *MMWR Morb Mortal Wkly Rep*. 2008;57:799–801.
91. Peters CJ. Lymphocytic choriomeningitis virus: an old enemy up to new tricks. *N Engl J Med*. 2006;354:2208–2211.
92. Amman BR, Pavlin BI, Albarino CG, et al. Pet rodents and fatal lymphocytic choriomeningitis in transplant patients. *Emerg Infect Dis*. 2007;13:719–725.
93. Schmitz H, et al. Monitoring of clinical and laboratory data in two cases of imported Lassa fever. *Microbes Infect*. 2002;4:43–50.
94. Solbrig MV. *Lassa Virus and Central Nervous System Diseases*. Plenum; 1993:325–330.
95. Johnson KM, et al. Clinical virology of Lassa fever in hospitalized patients. *J Infect Dis*. 1987;155:456–464.
96. Demby AH, Chamberlain J, Brown DW, et al. Early diagnosis of Lassa fever by reverse transcription-PCR. *J Clin Microbiol*. 1994;32:2898–2903.
97. Trappier SG, et al. Evaluation of the polymerase chain reaction for diagnosis of Lassa virus infection. *Am J Trop Med Hyg*. 1993;49:214–221.
98. Douglas RG, Wiebenga NH, Couch RB. Bolivian hemorrhagic fever probably transmitted by personal contact. *Am J Epidemiol*. 1965;82:85–91.
99. Maiztegui JI. Clinical and epidemiological patterns of Argentine haemorrhagic fever. *Bull World Health Organ*. 1975;52:567–575.
100. Stinebaugh BJ, et al. Bolivian hemorrhagic fever: a report of four cases. *Am J Med*. 1966;40:217–230.
101. McKee KT Jr, Huggins JW, Trahan CJ, et al. Ribavirin prophylaxis and therapy for experimental argentine hemorrhagic fever. *Antimicrob Agents Chemother*. 1988;32:1304–1309.
102. Andrei G, De Clercq E. Inhibitory effect of selected antiviral compounds on arenavirus replication in vitro. *Antiviral Res*. 1990;14:287–299.
103. Price ME, Fisher-Hoch SP, Craven RB, et al. A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy. *BMJ*. 1988;297:584–587.
104. Barton LL, Budd SC, Morfitt WS, et al. Congenital lymphocytic choriomeningitis virus infection in twins. *Pediatr Infect Dis J*. 1993;12:942–946.
105. Ackermann R, Korver G, Turss R, et al. Prenatal infection with the virus of lymphocytic choriomeningitis: report of two cases]. *Dtsch Med Wochenschr*. 1974;99:629–632, [in German].
106. Wright R, Johnson D, Neumann M, et al. Congenital lymphocytic choriomeningitis virus syndrome: a disease that mimics congenital toxoplasmosis or cytomegalovirus infection. *Pediatrics*. 1997;100:E9.
107. Barton LL, Mets MB, Beauchamp CL. Lymphocytic choriomeningitis virus: emerging fetal teratogen. *Am J Obstet Gynecol*. 2002;187:1715–1716.
108. Childs JE, Glass GE, Ksiazek TG, et al. Human-rodent contact and infection with lymphocytic choriomeningitis and Seoul viruses in an inner-city population. *Am J Trop Med Hyg*. 1991;44:117–121.
109. Briggiler AM, Enria DA, Maiztegui JI. Argentine hemorrhagic fever in pregnant women. *Medicina (B Aires)*. 1990;50.
110. Damonte EB, Coto CE. Treatment of arenavirus infections: from basic studies to the challenge of antiviral therapy. *Adv Virus Res*. 2002;58:125–155.
111. Andrei G, De Clercq E. Molecular approaches for the treatment of hemorrhagic fever virus infections. *Antiviral Res*. 1993;22:45–75.
112. McCormick JB, King IB, Webb PA, et al. Lassa fever: effective therapy with ribavirin. *N Engl J Med*. 1986;314:20.
113. Barry M, Russi M, Armstrong L, et al. Treatment of a laboratory-acquired Sabia virus infection. *N Engl J Med*. 1995;333:294–296.
114. Kilgore PE, Peters CJ, Mills JN, et al. Prospects for the control of Bolivian hemorrhagic fever. *Emerg Infect Dis*. 1995;1:97–100.
115. Borio L, Inglesby T, Peters CJ, et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA*. 2002;287:2391–2405.
116. Mackenzie RB. Epidemiology of Machupo virus infection. I. Pattern of human infection, San Joaquin, Bolivia, 1962-1965. *Am J Trop Med Hyg*. 1965;14:808.
117. Dykewitz CA, Dato VM, Fisher-Hoch SF, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. *JAMA*. 1992;267:1349–1353.
118. Keenlyside RA, McCormick JB, Webb PA, et al. Case-control study of *Mastomys natalensis* and humans in Lassa virus–infected households in Sierra Leone. *Am J Trop Med Hyg*. 1983;32:829–837.
119. Monath TP. Lassa fever: review of epidemiology and epizootology. *Bull World Health Organ*. 1975;52:577.
120. Peters CJ, Kuehne RW, Mercado R, et al. Hemorrhagic fever in Cochabamba, Bolivia, 1971. *Am J Epidemiol*. 1974;99:425–433.
121. Centers for Disease Control and Prevention. Interim guidance for managing patients with suspected viral hemorrhagic fever in U.S. hospitals. *MMWR Morb Mortal Wkly Rep*. 2005;http://www.cdc.gov/ncidod/dvrd/spb/pdf/vhf-interim-guidance.pdf.
122. Mitchell SW, McCormick JB. Physicochemical inactivation of Lassa, Ebola, and Marburg viruses and effect on clinical laboratory analyses. *J Clin Microbiol*. 1984;20:486.
123. Maiztegui JI, et al. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. *J Infect Dis*. 1998;177:277–283.

168 Human T-Cell Leukemia Viruses (HTLV-1, HTLV-2)

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SHORT VIEW SUMMARY

Definition

- Human T-cell leukemia virus (HTLV) is a human retrovirus of the subfamily Retroviridae and genus *Deltaretrovirus*.
- It causes chronic human infection by integration of proviral DNA into the somatic DNA of host T lymphocytes, with expansion of infectious burden by proliferation of infected lymphocytes more importantly than by production of free virus particles.
- To date, four types of the virus have been discovered: HTLV-1 and HTLV-2 infections are estimated in millions of humans, and HTLV-3 and HTLV-4 have occurred in only a few isolates since their discovery in the past decade.
- HTLV-1 causes adult T-cell leukemia-lymphoma (ATL), and both HTLV-1 and HTLV-2 cause HTLV-associated myelopathy (HAM).

Epidemiology

- HTLV-1 is endemic to central Africa, the Caribbean basin, parts of South America, and southwestern Japan, and in other discrete geographic areas. There is limited genetic variation and close homology to primate T-lymphotropic viruses, suggesting several instances of cross-species transmission, likely by bites from apes and monkeys sustained by humans.
- HTLV-2 is endemic to Amerindian tribes in North, Central, and South America and to Central African pygmies. It is hyperendemic among injection drug users in North America and Europe.
- Both HTLV-1 and HTLV-2 are transmitted by mother-to-child transmission predominantly by breastfeeding, by sexual transmission, and by parenteral infection.
- In populations in which they are endemic, both retroviruses show an increased prevalence in older individuals, and prevalence is frequently higher in females than in males.

Diagnosis and Pathogenesis

- HTLV antibodies may be detected with screening enzyme immunoassays and supplemental Western blot/recombinant immunoblot, with differentiation of HTLV-1 from HTLV-2 through use of type-specific antigens in supplemental assays.
- Polymerase chain reaction (PCR) assay to detect HTLV-1 or HTLV-2 proviral DNA in peripheral blood mononuclear cells may be performed if serology is inconclusive. PCR assay is also useful in detecting infection in infants carrying passive maternal antibodies.
- Most infected individuals are asymptomatic. Two percent to 4% of those infected with HTLV-1 will develop ATL, characterized by monoclonal integration of HTLV-1 provirus into a T-lymphocytic malignancy.
- One percent to 4% of those infected with either HTLV-I or HTLV-2 will develop HAM, a spastic paraparesis affecting predominantly the lower limbs and hyperactive bladder in a person infected with HTLV.

Therapy

- No treatment is currently recommended for persons with asymptomatic HTLV-1 or HTLV-2 infection. Periodic follow-up should include medical history, physical examination, and complete blood cell count to detect ATL or HAM.
- The lymphoma type of ATL is often treated initially with vincristine, cyclophosphamide, prednisolone, and doxorubicin with the addition of methotrexate, more complex 9- and 10-drug regimens. These have shown some success without prolonging long-term survival. Chronic, smoldering, and leukemia types of ATL often respond better to a combination of zidovudine and interferon-α.
- Therapy for HAM is currently unsatisfactory. Corticosteroids may produce improvement, but side effects limit the duration of therapy. Interferon-α and interferon-1β have been used with some success.

Prevention

- To interrupt mother-to-child transmission, HTLV-infected pregnant women in middle- and high-income countries should not breastfeed their infants if this can be accomplished safely.
- The use of condoms should be recommended to prevent sexual transmission.
- Injection drug users with HTLV infection should be instructed not to share needles.
- The US Food and Drug Administration recommends HTLV testing of all donated blood but not currently of donated organs. No vaccine is currently available.

Human T-cell leukemia virus type 1 (HTLV-1), the first recognized human retrovirus, was described in 1979 by Poiesz and coworkers,[1,2] who isolated retroviral particles with type C morphology budding from fresh cultured lymphocytes of a 28-year-old American man with a diagnosis of cutaneous T-cell lymphoma similar to cases seen in persons residing in Caribbean countries. Soon thereafter, Yoshida and associates[3] and Watanabe and colleagues[4] similarly isolated a new retrovirus from cases of adult T-cell leukemia-lymphoma (ATL), which was common in southwestern Japan at that time, and the two isolates were subsequently determined to be the same virus. The long-standing search for a human homologue to cancer-causing retroviruses of animals, first discovered at the beginning of the 20th century, ended at a time when most researchers had abandoned this quest and focused instead on viral transforming genes that occur as oncogenes in human tumors. HTLV type 2 (HTLV-2) was identified 2 years later from a case of variant T-cell hairy cell leukemia and has a different epidemiology and disease profile compared with HTLV-1.[5] More recently, rare isolates of two new HTLV types have been found among central African hunters and were designated HTLV-3 and HTLV-4 in 2005.[6,7] The techniques used to isolate and characterize these viruses provided the intellectual and technical basis for the discovery of the human immunodeficiency virus (HIV) in 1983, which was shown to be the cause of acquired immunodeficiency syndrome (AIDS) in 1984.

Within the taxa of DNA and RNA reverse-transcribing viruses, the HTLV viruses, along with bovine leukemia virus, are classified in the subfamily Retroviridae within the genus *Deltaretrovirus* (formerly *Oncovirus*).[8] The oncogenic properties of these viruses and their molecular

structure distinguish them from retroviruses HIV type 1 (HIV-1) and HIV type 2 (HIV-2), which are members of the genus *Lentivirus*. Both oncoviruses and lentiviruses are capable of prolonged asymptomatic infection.[8] In vitro, however, HIV-1 and HIV-2 have cytopathic effects on human T cells, whereas HTLV-1 and HTLV-2 are capable of transforming T cells, resulting in immortalized cell lines.

HTLV-1 has been shown to be associated with ATL and with a unique form of progressive neurological disease known as HTLV-associated myelopathy (HAM), also known as tropical spastic paraparesis (TSP). HTLV-2 is associated with HAM but is not known to cause leukemia or lymphoma. Although most HTLV-1 and HTLV-2 infections are asymptomatic, chronic infection with either retrovirus may cause other, more unusual immunologic and infectious complications.[9]

STRUCTURE AND MOLECULAR ORGANIZATION

The HTLV viruses are approximately 100 nm in diameter with a thin electron-dense outer envelope and an electron-dense, roughly spherical core (Fig. 168.1). Their genomic structure is illustrated in Fig. 168.2. The total provirus genome consists of roughly 9000 nucleotides with two flanking identical sequences. Long terminal repeats (LTRs) at the 5′ and 3′ ends of the genome are organized into three identical regions (U5, R, U3) that control gene expression through the requisite signals: enhancer, promoter, transcription initiation, and transcription termination and polyadenylation. Overall, there is 65% nucleotide homology between sequenced HTLV-1 and HTLV-2 isolates, 60% between HTLV-1 and HTLV-3,[10] and 62% to 71% between HTLV-4 and HTLV-1, HTLV-2, and HTLV-3, respectively.[11] Retroviral genes generally code for large overlapping polyproteins that are processed by a virally encoded protease and cellular proteases into functional peptide products. The HTLV viruses share with other replication-competent retroviruses the main genomic open reading frames (ORFs) of *gag* (group-specific antigen), *pro* (protease), *pol* (polymerase), and *env* (envelope) encoding for structural and enzymatic proteins (Table 168.1). However, unlike other vertebrate leukemia viruses, these deltaretroviruses have an additional region called *pX*. In HTLV-1, the *pX* region has six ORFs. Five of these reside on the plus strand, and the sixth, HBZ, resides on the minus strand. Of these six *pX* genes, three (*tax, rex,* and *HBZ)* have been implicated as crucial in sustaining HTLV infection by modulating replication and expression of regulatory proteins. As shown in Fig. 168.2, two overlapping reading frames are involved in the expression of both of these gene products translated from a doubly spliced messenger RNA (mRNA) involving the initiation codon from *env* and the remaining sequences from the *pX* region. In addition, ORFs encoding APH-2, APH-3, and APH-4 (antisense protein of HTLV) transcribe from the minus strand of the 3′ LTR in HTLV-1, HTLV-2, HTLV-3, and HTLV-4, respectively.[12] The detailed roles of these proteins are still being elucidated but may involve transcriptional regulation.

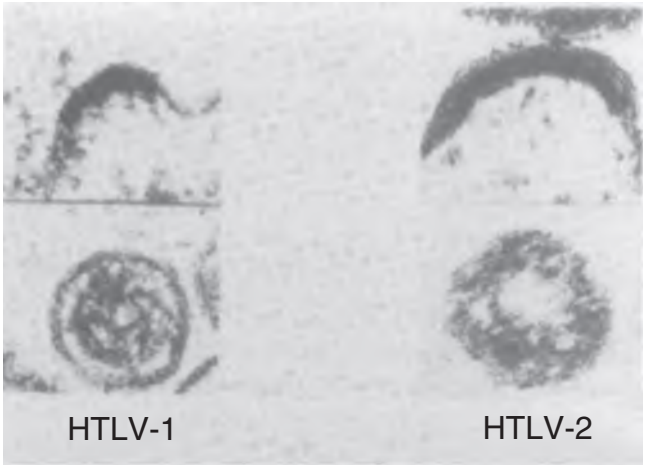

FIG. 168.1 Electron micrographs of human T-cell leukemia virus type 1 (HTLV-1) and HTLV-2. HTLV-1 and HTLV-2 have a diameter of approximately 100 nm. The budding particles are shown for each virus *(top)* and the mature virion *(bottom).* Both types of viruses have a roughly spherical electron-dense core.

gag, pro, pol, and *env*

The *gag* gene encodes structural proteins of the nucleocapsid, capsid, and matrix, also named p15, p24, and p19 GAG proteins, respectively. The *pro* gene slightly overlaps *gag* and *pol* and encodes protease, which cleaves GAG and GAG/POL polypeptides into proteins of the mature virion. The *pol* gene encodes the enzymes reverse transcriptase, which generates a double-stranded DNA from the RNA genome, and integrase, which integrates viral DNA into the host-cell chromosomes. The polymerase region contains the largest ORF in the HTLV genome, potentially able to encode an 896–amino-acid product for HTLV-1 and a 982–amino-acid product for HTLV-2. The polymerase genes of HTLV-1 and HTLV-2 share only 56% homology based on their predicted amino-acid sequences. The *env* gene encodes the major components of the viral coat: the surface glycoprotein of 46-kDa (gp46) and the transmembrane glycoprotein of 21-kDa (gp2l). Although HTLV-1, HTLV-2, HTLV-3, and HTLV-4 share the same overall genetic organization, they show some diversity at the nucleotide level, exhibiting a variable degree of amino-acid homology between viral capsid and envelope proteins.

Production of the GAG proteins derives from the translation of the full-length mRNA, which yields a large precursor polypeptide that is subsequently cleaved by the virally coded protease. POL proteins are expressed as polyproteins, both GAG-PRO and GAG-PRO-POL, and production is mediated by ribosomal frameshifts between *gag* and *pro* ORFs. For the POL proteins, production depends on translation made possible when the stop codon of the *gag* gene is bypassed, leading to a large polypeptide including GAG- and POL-related proteins, which are subsequently cleaved into functional proteins by the viral protease. Production of the ENV surface and transmembrane proteins involves translation of a spliced message (see Fig. 168.2), which results in an envelope precursor cleaved into the subunits. The precursor proteins have characteristic molecular weights that Western blot (WB) analysis can detect immunologically.

TAX

HTLV-1 TAX is a 40-kDa protein (p40), and HTLV-2 TAX is a 37-kDa protein (p37).[13,14] These proteins localize primarily to the nucleus of

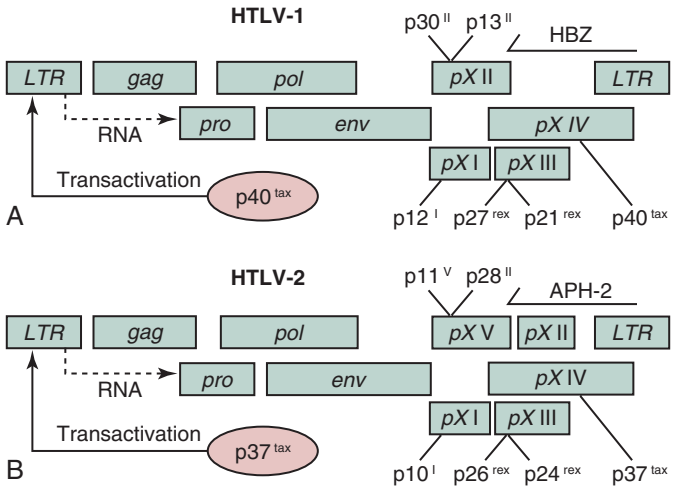

FIG. 168.2 Genomic structures of human T-cell leukemia virus type 1 (HTLV-1) and HTLV-2. (A) HTLV-1. (B) HTLV-2. *APH-2,* Antisense transcribed antisense protein of HTLV-2 gene involved in transcription regulation; *env,* envelope gene; *gag,* group-specific antigen whose products form the skeleton of the virion (matrix, capsid, nucleocapsid, nucleic acid–binding protein); *HBZ,* antisense transcribed HTLV-1 basic zipper protein involved in cell proliferation; *LTR,* long terminal repeat; *pol,* gene for reverse transcriptase and integrase; *pro,* gene for protease; *rex,* viral regulatory gene involved in promoting genomic RNA production; *tax,* transactivator gene.

TABLE 168.1 Major Structural and Regulatory Proteins of HTLV-1

VIRAL GENE	GENE PRODUCT (PROTEIN SIZE [kDa])	FUNCTION
5′ *LTR*		Regulation of viral gene expression and regulation of viral expression
5′ *LTR* and 3′ *LTR*		Integration points for provirus into host genome
hbz	HBZ	*hbz* mRNA is found in ATL tumor tissue and is responsible for lymphocyte proliferation
		HBZ protein prevents or suppresses Tax-mediated viral transcription
gag	p15	Nucleocapsid (NC) is a small basic protein found in the virion in association with genomic RNA
gag	p19	Matrix (MA) protein is myristoylated and anchored in the plasma membrane
gag	p24	Capsid (CA) protein forms the major internal structural feature of the core shell of the virion
gag	p53	Gag precursor
pro	Protease	Cleaves GAG precursor into CS, MA, NC, and Pol precursor in reverse-transcriptase and integrase proteins
pol	Integrase	Integrates viral DNA into the host-cell genome
pol	Reverse transcriptase	Reverse transcriptase generates a double-stranded DNA from the single-stranded RNA genome
env	gp21 and gp46	Envelope transmembrane and surface glycoproteins
pX	TAX (p40)	Transactivator that enhances transcription of viral promoter and alters transcription from cellular gene promoters
pX	REX (p27)	Regulator of unspliced and singly spliced viral mRNA transport from the nucleus to the cytoplasm
pX	p12[I]	Downregulates major histocompatibility complex class I trafficking to the surface to evade CTLs; elevates cytoplasmic calcium, increases T-cell activation, increases NFAT activation, enhances LFA-1–mediated T-cell adhesion, and activates STAT5b
pX	p8	Cleavage product of p12; increases formation of intercellular conduits and viral transmission
pX	p30[II]	Interferes with TLR4 signaling, alters cell cycle and DNA repair, and promotes viral latency by interfering with *tax/rex* RNA export
	p13[II]	Induces apoptosis; binds to and interferes with *tax* transactivation

ATL, Adult T-cell leukemia; *CTL,* cytotoxic T lymphocytes; *LFA-1,* lymphocyte function–associated antigen 1; *NFAT,* nuclear factor of activated T cells; *TLR4,* Toll-like receptor 4.

infected cells, although small amounts of TAX have been found in the cytoplasm. The TAX proteins are responsible for enhanced transcription of viral and cellular gene products and are essential for transformation of human T lymphocytes.[15] The TAX viral regulatory protein for HTLV-1, like its counterpart TAT of HIV-1, plays an important role in promoting viral growth and disease pathogenesis. Both promote *trans*-acting, transcriptional activation of the *LTR,* but the effect of TAX appears to be mediated via expression of cellular growth factors that are abundantly activated by TAX through its *trans*-activation properties. This *trans*-activation of cellular genes by TAX not only facilitates viral replication but also has emerged as a cofactor in disease pathogenesis. The *tax* gene is responsible for the *trans*-activation of virus transcription via *tax*-responsive elements of a number of regulatory enhancers, such as the 21-bp enhancer, the nuclear factor-κB (NF-κB) binding site, and serum-responsive element. Such promoter interactions lead to the activation of a number of cellular genes, such as those encoding interleukin (IL)-2 and the IL-2 receptor (IL-2R), which promote cell proliferation. In addition, TAX activates the proto-oncogenes *fos* and *erb*, and the gene for granulocyte-macrophage colony-stimulating factor, an array of early response genes, the human lymphotoxin gene, and parathyroid hormone–related protein gene, whereas it *trans*-represses the β-polymerase gene. Overproduction of interferon (IFN)-γ via this pathway has been implicated in promoting the chronic inflammation that characterizes diseases such as HAM.[16] The mechanism by which TAX interacts with a variety of cell regulatory elements involves nuclear regulatory elements (the NF-κB pathway). Both Tax-1 and Tax-2 have been found to inhibit expression of certain tumor suppressor genes in vitro, but because HTLV-2 has not been shown to be oncogenic, the effect or implication of this has not been determined in vivo.

HBZ and APH

The antisense proteins HBZ in HTLV-1 and APH in HTLV-2, HTLV-3, and HTLV-4 may also be involved in HTLV pathogenesis, with effects on proviral load and transcriptional regulation. Analyses of T-cell lines transfected with mutated *hbz* genes showed that *hbz* promotes T-cell proliferation in its RNA form, whereas HBZ protein suppresses TAX-mediated viral transcription through the 5′ LTR.[17] Thus far, HBZ has been shown to inhibit TAX-mediated activation of the HTLV-1 LTR, activate cellular transcription, and promote T-lymphocyte proliferation, whereas all APH proteins have demonstrated the ability to repress TAX-mediated viral transcription.[12,18–21] Both HBZ protein and mRNA are required for the propagation of virus and/or replication of infected T cells in vivo, as demonstrated in a humanized mouse model.[22] In HTLV-2, although APH-2 is associated with higher proviral load, it has not been shown to promote cell proliferation or lymphocytosis.[21,23] Recent work has demonstrated that the HBZ protein and HBZ mRNA act in concert to induce cellular proliferation through modulation of the apoptotic process,[24] stimulation of telomerase,[25] and suppression of a T-cell–specific protein (THEMIS).[26] In addition, the ability of the HBZ protein to bind a tumor suppressor (Rb) demonstrates possible oncogenic pathogenesis.

REX

The *rex* gene of HTLV-1 and HTLV-2 encodes two protein species in each virus. In HTLV-1, a 27-kDa protein (p27) and a 21-kDa protein (p21) seem to result from the use of alternative initiator methionine codons. In HTLV-2, however, a 26-kDa protein appears to be formed by phosphorylation of a serine residue in a 24-kDa protein.[27] Unlike the products of the *tax* gene, REX does not directly regulate RNA transcription, but instead appears to act chiefly at a posttranscriptional level to regulate viral gene expression. REX (regulator of expression of virion proteins for HTLV) stabilizes viral mRNA and is essential for export of full-length *gag/pol* and single-spliced *env* mRNA from the nucleus to the cytoplasm.[28] REX localizes to the nucleus and specifically to the nucleoli of infected cells.[29] Phosphorylated REX binds with high affinity to *cis*-acting RNA sequences, called REX-response elements (RxRE), in the viral mRNA.[30–32] This interaction facilitates the export of mRNA. REX binding may also inhibit mRNA splicing by preventing early steps in spliceosome assembly.[33] As a consequence of the accumulation of REX in the cell, there is an accumulation of unspliced and

single-spliced mRNA, favoring the production of structural proteins (GAG and ENV). This is accompanied by a decrease in the levels of double-spliced mRNA encoding TAX and REX. REX accumulation may also inhibit TAX, thus slowing viral transcription.[34] A fine balance between TAX and REX expression and function may dictate the rate of viral replication within infected cells.

Other Proteins Encoded by the *pX* Region

pX ORF I, produced by a double-splicing mechanism similar to that of the gene products of *pX* ORF III and IV, codes for a hydrophobic 12-kDa protein, $p12^{I}$, and *pX* ORF II results in the production of two nuclear proteins, $p13^{II}$ and $p30^{II}$.[35] In addition to activating nuclear factor of activated T cells,[36] $p12^{I}$ localizes in the endoplasmic reticulum and *cis*-Golgi apparatus and elevates cytoplasmic calcium that is antecedent to T-cell activation and essential for establishing persistent infection.[37] Other major structural and regulatory proteins of HTLV-1 are summarized in Table 168.1. The gene products of *pX* ORF I and II also appear to affect cell proliferation and modulate host immune responses to HTLV-1 infection. TAX and the ORF I and II gene products may play an integral role in the pathogenesis of HTLV-associated diseases through their effects on cyclins, which regulate cell growth.

BIOLOGY

The replication strategy of HTLVs involves a life cycle typical of all members of the Retroviridae (Fig. 168.3), whereby the RNA genome undergoes reverse transcription into a DNA provirus that integrates into the host genome. Subsequently, new virions are produced via this integrated DNA template under the regulation of viral regulatory genes. The HTLV-1 receptor was determined to be the ubiquitous glucose transporter GLUT-1,[38] consistent with the infection by HTLV-1 of a wide range of cells in vitro, including endothelial cells and fibroblasts.[39] In addition, a number of animal species can be infected either experimentally (mice, rats, rabbits, and New World primate species) or naturally (Old World primates).[40] The natural host range for HTLV is therefore humans and Old World nonhuman primates.

Although HTLV-1 can infect a number of different cell types in vitro, its growth and propagation in vivo are supported mainly by $CD4^{+}$ cells and, to a lesser extent, $CD8^{+}$ cells, as determined with real-time quantitative polymerase chain reaction (PCR) assay performed on flow cytometry–sorted T cells from patients with HAM.[41] In contrast, HTLV-2 preferentially infects $CD8^{+}$ cells over $CD4^{+}$ cells.[42] HTLV-1 also infects dendritic cells, although different subsets of dendritic cells encountered by HTLV-1 during primary infection may either favor or inhibit subsequent infection of CD4+ lymphocytes.[43]

Clonal expansion of infected lymphocytes, rather than viral replication and infection of new lymphocytes, has been demonstrated as the primary mechanism of viral expansion within chronically infected humans, especially in HTLV-1.[44] Limited numbers of large clones have been found in HTLV-1 carriers and in patients with HAM/TSP,[45] and the relationship between number and size of clones may be related to mode of transmission and to disease pathogenesis.[46,47]

Based on epidemiologic data demonstrating that HTLV-1 transmission is strongly cell associated and through in vitro studies in which cocultivation is required for efficient infection of target cells, transmission of HTLV is mediated by live cells and not via cell-free body fluids. For this reason, HTLV-1 is not an easily transmitted virus, and universal biohazard precautions are adequate for the inactivation of potentially infectious blood or bodily secretions. Procedures that remove or kill lymphocytes, such as leukoreduction or refrigerated storage of blood products and freeze/thawing of expressed breast milk, have been shown to reduce but not eliminate the risk for HTLV-1 transmission.[48,49,50]

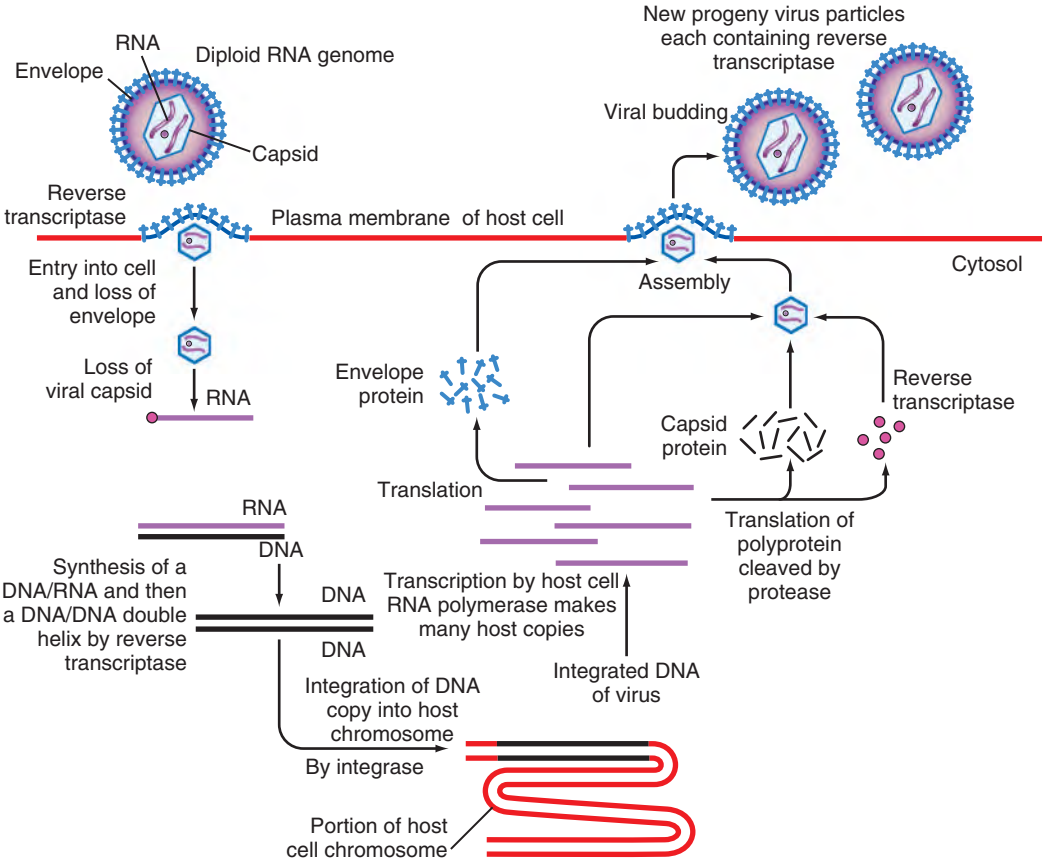

FIG. 168.3 Life cycle of human T-cell leukemia virus type 1 (HTLV-1). Virus infection involves initial binding to cell surface of target CD4 cell, uncoating, and release of viral genetic material. Virally encoded reverse transcriptase creates a DNA copy (provirus) that is integrated into the host genome under the influence of viral integrase. Viral replication involves production of both genomic RNA and polyproteins that are cleaved by the viral protease, resulting in virion assembly at the cell surface. See text for details. *(Modified from Alberts B, Bray D, Johnson A, et al, eds.* Essential Cell Biology: An Introduction to the Molecular Biology of the Cell. *New York: Garland Science Publishing; 2004.)*

Because HTLV-1 is highly cell associated, the means for viral attachment are not well characterized, but similar to other retroviruses, fusion of the virion with the cell membrane results in uncoating of the diploid RNA genome of the virus (see Fig. 168.3). Igakura and coworkers[51] found that additional cell surface–adhesion proteins and cell-cell contacts/interface (virologic synapse) are important for facilitating virus transmission. Cytoskeletal reorganization in the infected cells and segregation of virus particles to the interface between the infected and uninfected cells can be observed with immunofluorescence microscopy.[51,52,53]

Once in the cell, the virally encoded RNA-dependent DNA polymerase (reverse transcriptase) complexed to the genomic RNA of the virus transcribes viral RNA into double-stranded DNA. This double-stranded viral complementary DNA (cDNA) is transported to the nucleus as a ribonucleoprotein complex that includes the p24 capsid protein, and integrase and reverse transcriptase. Once there, the cDNA, through a complex process mediated by the viral integrase, is inserted into the host genome. The genomic integration of HTLV-1 establishes a lifelong infection and is integral to both the virus replication cycle and amplification of provirus.

Elements in the viral LTR are essential to integration and replication, because they form the sites for covalent attachment of the provirus to cellular DNA and provide important regulatory components for transcription. Additional key regulatory elements of HTLV are *tax,* which activates transcription of the viral genome, and *rex,* which modulates the processing of the viral RNA expressing unspliced forms of the viral mRNA. When the DNA provirus is expressed (i.e., transcribed by a cellular RNA polymerase), viral genomic RNA, mRNA, and, subsequently, viral proteins are made by the cell. Under the influence of REX, which stabilizes viral mRNAs and regulates their splicing and transport, new genomic RNA is assembled at the cell membrane and packaged for release. During the budding process, the envelope incorporates some of the cell's lipid bilayer, producing an infectious virion of about 100 nm (see Figs. 168.1 and 168.3).

LABORATORY DETECTION

Because HTLV-1 and HTLV-2 cause chronic infections and stimulate antibody production in most infected hosts, laboratory diagnosis can rely on detection of virus by culture of lymphocytes, anti-HTLV antibody, or HTLV genetic sequence by nucleic acid testing. Each approach has strengths and limitations, and diagnosis needs to be tailored to the specific clinical or epidemiologic setting.

Virus Isolation

Direct detection of virus through culture is intensive, expensive, and time-consuming; often several weeks are required for results to become available, and even then the result may be negative in infected persons. It is therefore impractical for clinical diagnosis in most clinical situations and is reserved for research. The ability to culture retroviruses has been improved by cocultivation of patients' T cells with human peripheral blood mononuclear cells (PBMCs) that have been stimulated in vitro with mitogens (e.g., phytohemagglutinin) and growth factors (e.g., IL-2), and by the removal of patients' $CD8^+$ suppressor cells from the coculture. The number of infected cells present in the blood of an infected individual is generally relatively low; mean proviral loads are 3.28 $\log_{10}$ copies per million PBMCs (range, 0.5–5.3) for HTLV-1 and 2.60 $\log_{10}$ copies per million PBMCs (range, 0.05–5.95) for HTLV-2.[54] The ability to isolate HTLV is dependent on viral load, immune status, and stage of disease. In infants and children, the small volume of blood available for culture and low viral load make virus isolation especially challenging.

Serologic Assays and Antigen Detection

The most common test for HTLV-1 and HTLV-2 infection is detection of antibody. Varieties of techniques are used to detect antibodies to HTLV-1. Because resolution of HTLV infection has not been described, confirmed HTLV-1 antibody positivity can be interpreted as infection with HTLV provirus. Samples are first screened with one of several assays that use whole-virus lysates or recombinant HTLV-1 and HTLV-2 antigens. The most widely used assay for the detection of HTLV-1 in the United States is the enzyme immunoassay (EIA), which uses whole disrupted virus and recombinant antigens.[55] This assay has demonstrated high sensitivity and specificity in the clinical setting but does not discriminate between HTLV-1 and HTLV-2 because of cross-reactive antibodies. Nevertheless, because false-positive results are common when EIAs are used in low-prevalence populations such as blood donors, repeating the EIA and retesting repeatedly reactive EIAs with a different supplemental test is strongly recommended before informing the patient.

For HTLV-1 and HTLV-2 infection, a combination of a screening EIA followed by a confirmatory WB is the standard approach, although some investigators and blood banks prefer alternative strategies incorporating a particle agglutination test for screening or an immunofluorescence assay for confirmation.[56] In December 2014, a test using a WB recombinant envelope protein became the first HTLV-1/HTLV-2 confirmatory test to be approved by the US Food and Drug Administration (FDA).[57] The line EIA, or LIA, uses well-defined antigens derived from HTLV-1 and HTLV-2 immunodominant proteins that are either recombinant proteins or synthetic peptides, highly purified and fixed on a nylon membrane strip. The sequences are selected to allow the detection of antibodies with a wide specificity to all known isolates of the HTLV strains. The antigenicity exhibited by these proteins and peptides is either common to both HTLV-1 and HTLV-2 or type specific to one to allow confirmation and discrimination in a single assay. Two *gag* (p19-1/2, p24-1/2) and two *env* (gp46-1/2, gp21-1/2) bands are applied as non–type-specific antigens, which are used to confirm the presence of antibodies against HTLV-1/2. The type-specific antigens for HTLV-1 (*gag* p19-1, *env* gp46-1) and for HTLV-2 (*env* gp46-2) are applied to differentiate between HTLV-1 and HTLV-2 infections.[58]

The radioimmunoprecipitation assay, a more difficult procedure based on radiolabeled virus-infected whole cells, is more sensitive for ENV antibodies but rarely available. Confirmatory testing with an immunofluorescence assay is also possible, although this assay is not suited for high-throughput operations because it is labor-intensive and subjective. Titration to allow quantification of antibody is possible by testing serial dilutions of serum with EIA or particle agglutination or immunofluorescence assays.

In tropical countries, particularly Africa, repeatedly reactive EIA-reactive samples exhibit a high frequency of indeterminate WB results. Typically, these indeterminate sera show an HTLV GAG indeterminate profile: GAG p19, p26, p28, and p30 without p24 or ENV gp21 and gp46, which may be related to endemic *Plasmodium falciparum* infection.[59] To avoid overestimating the rate of HTLV-1/HTLV-2 seroprevalence in these regions, PCR assay may be useful.[60,61]

Nucleic Acid Detection

PCR assays have been developed to distinguish virus type and to quantify viral presence. In this assay, proviral DNA is amplified enzymatically and subsequently detected through use of a system of specific nucleotide primers and probes.[62] The limit of detection is approximately one infected cell per million PBMCs.[63] With the addition of a reverse-transcription step before amplification, the PCR assay has been used in the research setting to detect viral RNA in infected cells, which helps to identify actively replicating virus, although RNA has not been convincingly demonstrated in ex vivo plasma samples. Although exquisitely sensitive in the best laboratories, the PCR assay remains a research technique; it is under consideration for use as a screening assay as new technologies are evolving. PCR assay has proved valuable in enigmatic situations such as cases of suspected HTLV disease in the setting of a seroindeterminate WB result. If used with degenerate primers capable of detecting a variant of HTLV, the PCR assay can also reveal the presence of a new exogenous retrovirus.[59] However, the PCR assay has only occasional clinical usefulness in the diagnosis of HTLV infection in low-risk populations because it also is subject to false-positive results. With a low pretest probability (nonendemic area, no risk factors), individuals with positive EIA and indeterminate WB results have only a 1% to 2% probability of true HTLV infection, so the PCR assay is not recommended.[64] Conversely, the PCR assay is useful in the diagnosis of neonatal HTLV infection because passively transferred maternal antibody makes antibody testing unreliable.[65]

The PCR technique has also been useful to facilitate epidemiologic research studies.[62] Theoretically, virus-positive, antibody-negative individuals could be missed with antibody tests, and the true prevalence

of virus may be underestimated. In fact, several surveys using the PCR assay have not detected more than the occasional virus-positive, antibody-negative individual.[66–68] Quantitative real-time PCR has allowed the measurement of HTLV proviral DNA in PBMCs from infected individuals in the research setting but is not readily adaptable to large-scale screening methods.[44,54,63,69] Neither has proviral load been fully characterized as a useful prognostic marker, and these assays are not available clinically.[70,71,72]

SEROLOGIC EPIDEMIOLOGY

Geographic Distribution of Prevalence

Fig. 168.4 shows the different geographic distributions of HTLV-1 and HTLV-2. Although precise estimates of the global burden of HTLV are difficult, a review of worldwide prevalence of HTLV-1 has placed the estimate at 5 to 10 million persons.[73] The largest endemic area for HTLV-1 is most probably Africa, with seroprevalence ranging from 0.5% to 5.5%. Central Africa appears to be the most highly endemic, although seroprevalence surveys have not been done in many African countries and the studies that have been done focused primarily on blood donors or pregnant women. Surveys from the Ivory Coast, Ghana, Nigeria, Democratic Republic of Congo, Kenya, and Tanzania document that rates of HTLV-1 seropositivity are similar to those in the Caribbean region (5%–14%), but more and better data from sub-Saharan Africa are needed.[74,75]

In Asia, endemic clusters of HTLV-1 infection are present in southern Japan, the islands of the Ryukyu chain including Okinawa, and some isolated villages in the north of Japan among aboriginal Ainu populations; most of the seropositives in northern Japan are among immigrants from endemic areas in the south.[76,77,78] Rates of infection among persons older than 40 years exceed 15% in these areas, and in some highly endemic areas they reach 30% to 40% in adults older than 50 years.[73,77,78] Inhabitants of the People's Republic of China and Vietnam are largely free of infection,[79] whereas those living in Hong Kong and Korea have very low seroprevalence rates (0.0041% and 0.007%–0.25% in blood donors, respectively).[73,80] The rates in Taiwan are slightly higher, with 0.058% in blood donors[73] and 0.82% to 1.63% in adults older than 30 years.[81]

In Australia, HTLV-1 is highly endemic (>30%) in aboriginal groups of inland Australia but occurs at very low rates in white nonaboriginals.[82–84] HTLV-1 seroprevalence is 0.6% in New Caledonia, 1% to 3% in the Solomon Islands, and very high (>15%) in Papua New Guinea.[73,85,86]

In the Americas, a major endemic focus of HTLV-1 infection occurs in the Caribbean, where rates of seropositivity in Jamaica, Trinidad and Tobago, Barbados, Haiti, and the Dominican Republic range between 5% and 14%, most in older persons and those of African descent.[87,88] In Jamaica, varying rates of seropositivity occur in different regions, with the highest rates (10%) of positivity observed in the lowland, high-rainfall areas.[89] In Trinidad and Tobago, seropositivity (5%–14%) is restricted almost exclusively to persons of African descent, even though individuals of Indo-Asian ethnic background have shared a common environment for more than a century. Seropositivity is found more frequently in persons of lower socioeconomic class and those who lack formal education.[81,87,88,90,91] Men and women attending clinics for sexually transmitted infections have the highest rate of seropositivity (>15%).[91]

Low rates of seropositivity are present in Central America, including Panama, Honduras, Costa Rica, and Nicaragua.[73] Of note is the higher rate in the non-Mestizo population compared with the Mestizo ethnic population, especially in coastal areas, in Honduras (8.1% vs. 0.5%).[73] In the entirety of South America, HTLV-1 is considered endemic, but several areas are foci of high endemic rates, primarily in indigenous Amerindians (Peru, Colombia, Chile, and Brazil) and, occasionally, specific groups of African origin (French Guiana, Surinam, Guyana, Colombia, and Brazil).[73,92,93] Brazil is considered one of the highest areas of endemic HTLV-1 infection (especially in Bahia and the northeast), whereas rates are very low in Venezuela, Paraguay, and Argentina.[73,94]

In North America, large-scale screening of blood donors in the United States has documented rates of HTLV-1 or HTLV-2 of up to 0.3 to 0.4 per 1000. Of the donors who test HTLV seropositive, one-half to two-thirds are HTLV-2 infected.[95,96,97] In a significant proportion of HTLV-1–positive cases, the donor has either links to an endemic area or a history of risk behaviors, such as blood transfusion or multiple sexual partners.[95] Populations that migrated from Okinawa to Hawaii, from the Caribbean to the United States, and from the Caribbean to the United Kingdom are at risk for HTLV positivity, as are those who experience exposure through sexual contact or blood transfusion in viral endemic areas.[90,98] HTLV infection is rare in Canada; when it is found, it is usually in migrants from endemic areas. However, several studies have documented HTLV-1 infections in First Nations peoples in coastal British Columbia. Little information is available on HTLV prevalence in Mexico, but the few studies that have been done indicate a near absence of infection.[73]

In Europe, small numbers of HTLV-1 infections are detected among emigrants from endemic areas to the United Kingdom, France, Spain, and other countries.[99–101] Middle East survey results have been largely negative, with the exception of Iranian Jews from northeastern Iran (Mashhad) and emigrants from that area now residing in Israel and New York.[102,103] Surveys in southern India and Indonesia have identified some HTLV-1–infected individuals but not endemic foci; the Seychelles in the Indian Ocean are highly endemic for HTLV-1 (>15%).[104]

HTLV-2 has a more restricted distribution than HTLV-1, primarily occurring in the Americas and parts of West and Central Africa.[105,106,107] A major reservoir exists in injection drug users in the United States and southern Europe, with rates ranging from 10% to 15% and higher.[108,109] Amerindians residing in North, Central, and South America have varying rates of positivity for HTLV-2 (5%–30%). Pockets of infection are present among the Seminoles in south Florida, the Pueblo and Navajo in New Mexico, and the Athapaskan in northwestern United States and Canada but not among various tribes in Alaska.[110,111] In Central America, the Guaymi Indians residing in northeastern Panama near the Costa Rican

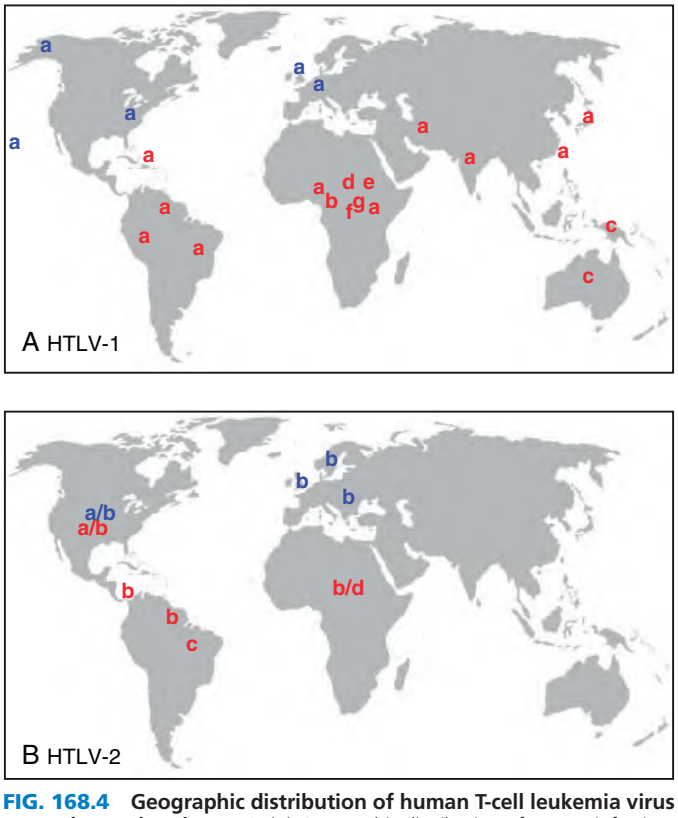

FIG. 168.4 Geographic distribution of human T-cell leukemia virus type 1 (HTLV-1) and HTLV-2. (A) Geographic distribution of HTLV-1 infection. HTLV-1–endemic populations are shown in *red,* and areas with sporadic cases of HTLV-1 often due to immigration from endemic areas are shown in *blue.* (B) Geographic distribution of HTLV-2 molecular subtypes 2a, 2b, 2c, and 2d. Areas with endemic HTLV-2–infected Native American and African populations are shown in *red,* and areas with HTLV-2 infection among intravenous drug users and their sexual partners are shown in *blue. (From Beilke MA, Murphy EL. The human T-lymphotropic leukemia viruses 1 and 2. In: Volberding PA, Palefsky J, eds.* Viral and Immunological Malignancies. *Hamilton, Ontario: BC Decker; 2006:328, 330.)*

border have high seropositive rates (>15%), but this does not hold true for the Guaymi living in southwest Panama. In South America, HTLV-2 is endemic in some indigenous Amazonian tribes of Peru and Brazil, and in the Chaco Indians of Argentina, with secondary spread to other population groups in those countries.[94] Finally, HTLV-2 has been identified in Africa, primarily among pygmy populations living in Central and Western rain forest areas.[106,112]

Demographic Patterns in Prevalence

HTLV-1 seroprevalence rates are strongly dependent on age and sex, with higher rates associated with older age and with female sex (Fig. 168.5).[76,88,113] The increasing prevalence with age may be due to the accumulation of infections over the lifetime of the individuals surveyed or birth cohort effect due to declining HTLV-1 seroprevalence over the past decades. The higher prevalence in females may be the result of more efficient male-to-female sexual transmission, as discussed later, or differences in sociodemographic or behavioral factors, such as duration of breastfeeding or frequency of condom use.

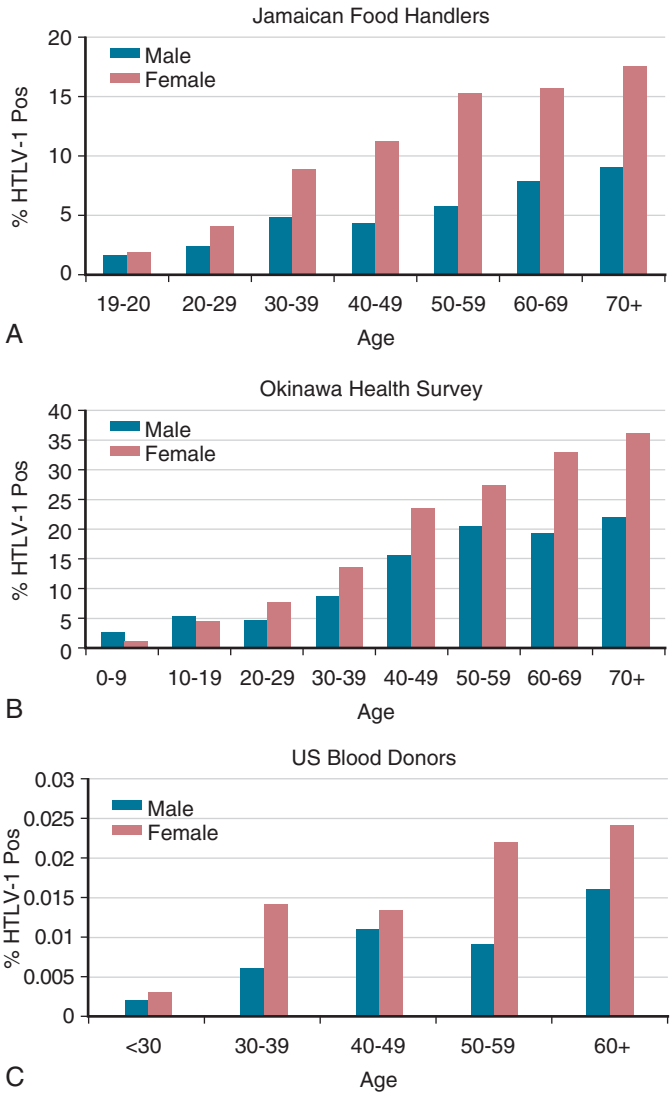

FIG. 168.5 Age- and sex-specific seroprevalence of human T-cell leukemia virus type 1 (HTLV-1) in several population groups. Graphs show the characteristic higher female prevalence and increasing prevalence with age. (A) Jamaicans employed in food-handling occupations (data from Murphy and colleagues[88]). (B) Participants in a community health survey in Okinawa, Japan (data from Kajiyama and colleagues[76]). (C) US blood donors with very low HTLV-1 seroprevalence (data from Murphy and colleagues[97]). *(From Beilke MA, Murphy EL. The human T-lymphotropic leukemia viruses 1 and 2. In: Volberding PA, Palefsky J, eds.* Viral and Immunological Malignancies. *Hamilton, Ontario: BC Decker; 2006:329.)*

For HTLV-2, there is also a characteristic age-dependent increase in seroprevalence in endemic Amerindian populations (Fig. 168.6).[114] In contrast, prevalence among US blood donors shows an HTLV-2 age-specific prevalence that peaks at age 40 to 50, consistent with a birth cohort effect due to injection drug use in the 1960s and 1970s, with secondary sexual transmission.[97]

Incidence

As might be expected from its relatively low infectivity, the incidence of HTLV-1 and HTLV-2 infections is quite low, even in endemic areas. Studies in large populations of repeated blood donors have measured seroconversions and thereby estimated incidence in the general population. Incidence rates in blood donors in HTLV-1–endemic Brazil and Japan were 3.6 and 3.8 per 100,000 person-years, respectively.[115,116] In contrast, studies in blood donors in the nonendemic United States ranged from 0.2 to 1.6 per 100,000 person-years.[117,118]

MOLECULAR EPIDEMIOLOGY

HTLV-1

Phylogenetic analysis has been used to classify HTLV-1 into seven major molecular and geographic subtypes: the Cosmopolitan subtype (a), with worldwide distribution, the Australo/Melanesian subtype (c), and five African subtypes (b, d, e, f, g)[119]; HTLV-1 isolates from different parts of the world show a high degree of nucleotide sequence conservation, in contrast to HIV-1 and HIV-2, in which considerable genomic variability occurs. Isolates of HTLV-1 from Japan, the West Indies, the Americas, and Africa share 97% or greater homology.[120–122] Even the most divergent HTLV-1c, isolated in Melanesia, is 92% homologous with the prototypic Japanese HTLV-1a isolate.[123] The majority of the nucleotide differences are single point mutations that do not correlate with specific disease patterns. Studies of the LTR by restriction fragment length polymorphisms

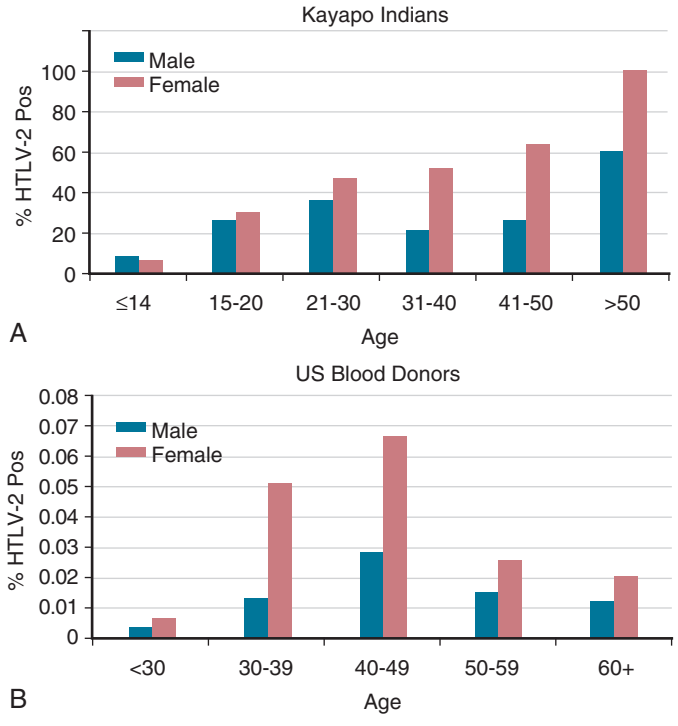

FIG. 168.6 Age- and sex-specific seroprevalence of human T-cell leukemia virus type 2 (HTLV-2). (A) Prevalence of HTLV-2 in the highly endemic Kayapo Indians of Brazil, consistent with ongoing mother-to-child and sexual transmission within a closed population (data from Maloney and colleagues[114]). (B) US blood donors with very low HTLV-2 seroprevalence show a pattern that suggests a birth cohort effect due to intravenous drug use in secondary sexual transmission in the 1960s and 1970s (data from Murphy and colleagues[97]). *(From Beilke MA, Murphy EL. The human T-lymphotropic leukemia viruses 1 and 2. In: Volberding PA, Palefsky J, eds.* Viral and Immunological Malignancies. *Hamilton, Ontario: BC Decker; 2006:332.)*