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147 Human Parvoviruses, Including Parvovirus B19V and Human Bocaparvoviruses

Kevin E. Brown

SHORT VIEW SUMMARY

Definition
- At least five different types of parvoviruses infect humans.
- Parvovirus B19 (B19V) can cause erythema infectiosum (slapped cheek disease or fifth disease), transient aplastic crisis, and pure red cell aplasia or fetal hydrops.
- Human bocaparvoviruses (HBoVs) can cause respiratory infections and may be associated with some cases of gastroenteritis.
- Human dependoparvovirus infections (adeno-associated viruses) are asymptomatic, and modified dependoparvoviruses are used as vectors for gene therapy.
- Disease associations with the human protoparvoviruses and Parv4 infection are not clear.

Epidemiology
- Parvovirus B19 (B19V) infection is a common infection in children and young adults. By age 15 years, 50% of children in America and Europe will have been infected and have immunoglobulin G against B19V.
- B19V is mainly spread through the respiratory route, although it may also be transmitted through blood and blood products.
- B19V infections in temperate climates are more common in late winter, spring, and early summer, with increased rates of infection every 3 to 5 years.
- HBoV infections are ubiquitous in young children, with most, if not all, children being infected with HBoV1 by age 6 years.

Diagnosis
- Although the slapped-cheek rash of B19V infection is classic, it is difficult to accurately diagnose outside of the context of an outbreak.
- Diagnosis of B19V rash is by detection of B19V immunoglobulin M in serum.
- Hematologic disease due to B19V can be diagnosed by detection of high-titer B19V DNA ($>10^6$ IU/mL) in blood samples.
- After infection, low levels of B19V DNA may be detected lifelong. Detection of low-level B19V DNA in samples therefore does not indicate recent or current infection.
- Similarly, long-term persistence of bocaparvovirus DNA in respiratory and fecal samples indicates that detection of viral DNA alone does not correlate with active infection. Respiratory HBoV1 infection should be diagnosed by detection of viral DNA in serum or serology, or both.

Therapy
- Treatment for all parvovirus infections is mainly symptomatic.
- Intravenous immunoglobulin can be used for treatment of chronic anemia or pure red aplasia due to high-titer B19V infection.

Prevention
- A vaccine for B19V is in development.

Parvum is Latin for "small," and the Parvoviridae are among the smallest known DNA-containing viruses that infect mammalian cells. The virions are nonenveloped particles about 22 nm in diameter with icosahedral symmetry. The Parvoviridae are divided into two subfamilies, Parvovirinae and Densovirinae, on the basis of their ability to infect vertebrate or invertebrate cells, respectively. The Parvovirinae are further subdivided into eight genera on the basis of their transcription map, their ability to replicate efficiently either autonomously or with a helper virus, and their sequence homology. The eight genera are *Protoparvovirus* (previously *Parvovirus*), *Dependoparvovirus, Erythroparvovirus, Bocaparvovirus, Amdoparvovirus, Aveparvovirus, Copiparvovirus,* and *Tetraparvovirus*.[1]

At least five different parvoviruses are known to infect humans. Parvovirus B19 (B19V) is the best characterized and is classified as a member of the *Erythroparvovirus* genus, of which it is the type species. The other human viruses are the human adeno-associated viruses (AAVs, or dependoparvoviruses), human bocaparvovirus (HBoV), human Parv4 virus, a member of the newly created *Tetraparvovirus* genus, and the human protoparvoviruses—human bufavirus (BuV), tusavirus (TuV), and cutavirus (CuV).[2]

PARVOVIRUS B19

Parvovirus B19 (B19V) was discovered in 1975 during evaluations of assays for hepatitis B surface antigen using panels of serum samples.[3] Sample 19 in panel B (hence B19) gave an anomalous result, a "false positive" in the relatively insensitive counterimmunoelectrophoresis assay, and when the precipitin line was excised, electron microscopy showed the presence of 23-nm particles resembling parvoviruses. Although originally labeled serum parvovirus-like particle or human parvovirus, in 1985, the virus was officially recognized as a member of the Parvoviridae, and the International Committee on Taxonomy of Viruses recommended the name B19V to prevent confusion with other viruses.

An association of B19V with significant clinical disease was not made until 1981, but it is now known that B19V infection has a wide variety of disease manifestations dependent on the immunologic and hematologic status of the host (Table 147.1). In normal immunocompetent children, B19V is the cause of erythema infectiosum (EI), also called fifth disease or "slapped cheek" disease, which is an innocuous illness with rash. On occasion, especially in women, fifth disease leads to an acute symmetrical polyarthropathy, which can mimic rheumatoid arthritis. In persons with underlying hemolytic disorders or increased erythropoiesis, or both, infection leads to a temporary failure of red blood cell production and transient aplastic crisis (TAC). In the immunocompromised host, persistent B19V viremia manifests as pure red cell aplasia (PRCA) and chronic anemia, and in the fetus, in which the immune response is immature, infection may lead to fetal death in utero, hydrops fetalis, or, rarely, the development of congenital anemia.

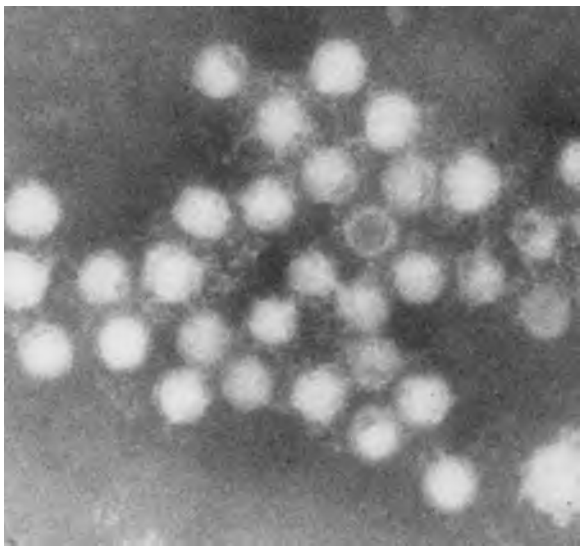

FIG. 147.1 Electron micrograph of parvovirus B19 particles showing icosahedral symmetry. *(Courtesy Dr. Anne Field.)*

TABLE 147.1 Disease Manifestations and Persistence of Parvovirus B19 Infection in Different Host Populations

DISEASE	ACUTE OR CHRONIC	HOST
Fifth disease	Acute	Normal children
Polyarthropathy syndrome	Acute or chronic	Normal adults
Transient aplastic crisis	Acute	Patients with increased erythropoiesis
Hydrops fetalis or congenital anemia	Acute or chronic	Fetus (<20 wk)
Persistent anemia	Chronic	Immunodeficient or immunocompromised patients

VIROLOGY

By electron microscopy, B19V particles have the typical parvovirus morphology (Fig. 147.1). Mature infectious particles have a molecular weight of 5.6×10^6 and a buoyant density in cesium chloride gradients of 1.41 g/mL. As a consequence of the lack of envelope and limited DNA content, B19V is resistant to physical inactivation. Although the virus can be inactivated by heat in low concentrations of protein,[4] the virus resists inactivation at 56°C for more than 60 minutes, and at high viral concentrations, it resists 80°C for 72 hours in clotting factor concentrates.[5] B19V is stable in lipid solvents such as ether and chloroform but can be inactivated by formalin, β-propiolactone, oxidizing agents, and γ-irradiation.

The B19V genome size is limited, consisting of a single strand of DNA of approximately 5596 nucleotides, with identical inverted, 365-nucleotide-long terminal repeat sequences at each end. The transcription map of B19V distinguishes it from other Parvovirinae. There is a single strong promoter at the far left side of the genome and unusual polyadenylation signals in the middle of the genome.[6] The three major viral proteins, one nonstructural (NS) protein and two capsid proteins, are produced by alternative splicing from the promoter and its accompanying leader sequence. The relative quantities of the major and minor capsid proteins are in part regulated by the presence of multiple upstream adenine-uridine-guanine (AUG) codons situated before the authentic transcription initiation codon.[7] In addition, there are transcripts for several smaller proteins of 7.5 and 11 kilodalton (kDa). Although the 11-kDa protein is known to be required for producing infectious virus, the role of the 7.5-kDa protein is unknown.[8]

The only unspliced transcript encodes the NS protein, a 78-kDa phosphoprotein.[9] Consistent with its role in viral propagation, the protein has DNA-binding properties and adenosine and guanosine triphosphatase activity.[10] Expression of the NS protein causes host cell death through induction of apoptosis.[11]

The B19V virion is an icosahedron consisting of 60 copies of the capsid proteins. Most of the capsid is VP2, a 58-kDa protein with 5% or less of the larger 84-kDa VP1 protein. VP1 protein differs from VP2 by an additional 227 amino acids at the amino terminus. Using genetic engineering techniques, the capsid proteins can be expressed in a variety of both mammalian,[12] insect,[13,14] and yeast[15] cell lines. Capsid proteins self-assemble in the absence of B19V DNA, and in these systems, protein expression leads to formation of recombinant empty capsids; VP1 is not required for capsid formation.

The atomic structure of both B19V VP2 empty capsids and infectious virus has been resolved.[16,17] The virion surface has a major depression encompassing the fivefold axis, similar to the canyon structure found in RNA-containing icosahedral viruses. In B19V capsids there is also a hollow cylindrical structure around the fivefold axes that appears to penetrate to the inside of the virion. The structural distribution of VP1 in the B19V capsid structure cannot be inferred from the crystallographic structures, but on the basis of antibody-binding and structural studies, in infectious B19V the VP1 unique region appears to be exposed on the viral surface adjacent to the fivefold axis cylinder.[17] It has been shown that the VP1 unique region of all parvoviruses, including B19V, has a phospholipase A_2 motif.[18] Infection studies with B19V and other parvoviruses show that this motif is required for viral infectivity.[18,19]

It is now recognized that there are three different genotypes, with approximately 10% variability at the DNA level between them.[20] Most of the B19V identified is genotype 1,[21] the original B19V genotype, and is distributed worldwide. Genotype 3 seems to be the predominant B19V genotype in Ghana, representing more than 90% of the sequences identified.[22] Genotype 2 has been primarily identified in tissues of older patients (born before 1973), suggesting that it may have circulated more frequently before the 1970s.[23,24] However, blood samples or donations containing high-titer genotype 2 are occasionally identified,[25] and genotype 2 and 3 sequences have been identified in blood and tissues from many different parts of the world,[26–29] suggesting a more widespread distribution than originally assumed. However, the true prevalence of these different genotypes is currently unknown.

Despite the differences in the DNA sequences, the capsid protein sequence is conserved between the different genotypes, and there is evidence for both serologic and cross-neutralization.[25,30,31]

PATHOGENESIS

B19V, like the other autonomous parvoviruses, is dependent on mitotically active cells for replication. However, B19V has a narrow target cell range and can be propagated efficiently only in human erythroid progenitor cells. The virus cannot be easily cultivated in the laboratory, apart from in primary erythroblasts.[32] Humans are the only known host of B19V, although primates have their own related erythroparvoviruses.[33]

In human erythroid cells derived from bone marrow, susceptibility to B19V increases with differentiation; the pluripotent stem cell appears to be spared, and the main target cells are erythroid progenitors (cells capable of giving rise to erythroid colonies in vitro) and CD36-positive erythroblasts.[32,34] Infection with B19V is cytotoxic[35] because of expression of the NS protein in infected cells.[11] Infected cultures are characterized by the presence of giant pronormoblasts, 25 to 32 μm in diameter, with cytoplasmic vacuolization, immature chromatin, and large eosinophilic nuclear inclusion bodies (Fig. 147.2). By electron microscopy, virus particles are seen in the nucleus and cytoplasmic membrane lining, and infected cells show marginated chromatin, pseudopod formation, and cytoplasmic vacuolation,[36] all of which are typical of cells undergoing apoptosis.

Erythroid specificity of B19V is, in part, due to the tissue distribution of the virus's cellular receptor globoside, also known as blood group P antigen.[37] P antigen is found on erythroid progenitors, erythroblasts, and megakaryocytes.[38] It is also present on endothelial cells, which may

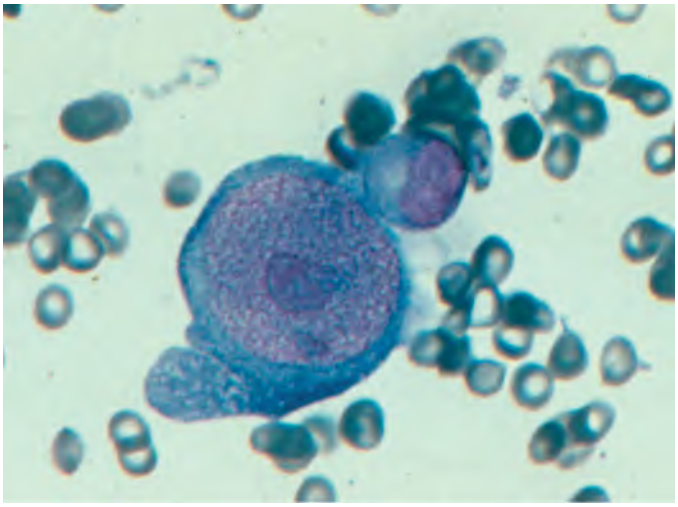

FIG. 147.2 Giant pronormoblast in patient with parvovirus B19 infection.

be targets of viral infection involved in the pathogenesis of transplacental transmission, possibly vasculitis, and the rash of fifth disease and on fetal myocardial cells. Rare individuals who genetically lack P antigen on erythrocytes are resistant to B19V infection, and their bone marrow cannot be infected with B19V in vitro.[39] However, although P antigen is required for infection, it is not sufficient for viral entry, and additional B19V receptors have been proposed as putative cellular receptors.[40–42] In addition, the erythroid specificity may also be modulated by specific erythroid cell transcription factors.

Studies in healthy volunteers showed that B19V infection led to an acute but self-limited (4–8 days) cessation of red cell production and a corresponding decline in hemoglobin level.[43] In patients with normal erythroid turnover, this short interruption of red cell production does not lead to anemia, but in patients with high red cell turnover related to hemolysis, blood loss, or other causes, the temporary failure of erythropoiesis can precipitate an aplastic crisis. The anemia improves as the immune response develops. In patients who are immunocompromised, infection may persist and produce chronic PRCA.

The infected fetus may suffer severe effects because red blood cell turnover is high and the immune response deficient. During the second trimester there is a great increase in red cell mass. Parvovirus particles can be detected by electron microscopy within the hematopoietic tissues of the liver and thymus.[44] B19V DNA and capsid antigen have been detected in the myocardium of infected fetuses,[45] and there is evidence that the fetus may develop myocarditis,[46] compounding the severe anemia and secondary cardiac failure. By the third trimester, a more effective fetal immune response to the virus probably accounts for the decrease in fetal loss at this stage of pregnancy.

The pathogenesis of the rash in EI and polyarthropathy is almost certainly immune complex mediated (Fig. 147.3). In volunteer studies these appeared when high-titer viremia was no longer detectable and coincident with a detectable immune response.[43] Similar findings have been reported in chronically infected individuals who received immunoglobulin therapy.[47] However, in vitro studies have shown that the B19V NS protein induces not only apoptosis in host cells but also activation of interleukin-6,[48] and the phospholipase motif in the VP1u region is functional[49,50]; both of these findings could contribute in vivo to the B19V-induced arthropathy.

EPIDEMIOLOGY

Prevalence and Incidence

B19V infection is common in childhood, and by age 15 years, approximately 50% of children have detectable immunoglobulin G (IgG) against B19V. Infection also occurs in adult life, and greater than 80% of elderly people have detectable antibody.[51] Women of childbearing age in the United States and Europe have an annual seroconversion rate of approximately 1%.[52,53] Studies in different countries (France, Germany,

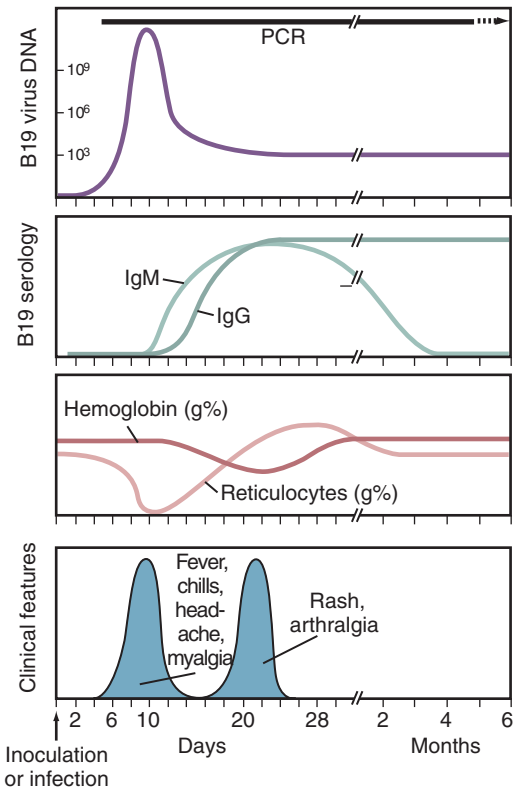

FIG. 147.3 Virologic, immunologic, and clinical courses after acute parvovirus B19 infection in a healthy, immunocompetent individual. *DNA,* Deoxyribonucleic acid; *PCR,* polymerase chain reaction; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M. *(From Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans.* J Infect Dis. *1985;152:257–265; and Patou G, Pillay D, Myint S, Pattison J. Characterization of a nested polymerase chain reaction assay for detection of parvovirus B19.* J Clin Microbiol. *1993;31:540–546.)*

Japan, United Kingdom, and United States) show similar patterns of seroprevalence, with significantly higher levels in parts of Africa and Papua New Guinea, with greater than 80% of 10-year-olds having detectable antibody.[54] In contrast, parts of Asia[55] and some isolated tribal populations have a much lower prevalence.[56]

Although antibody is prevalent in the general population, very high-titer viremia (>10^9 IU/mL) is rare: Approximately 1 per 20,000 to 1 per 40,000 units of blood during epidemic seasons contain high-titer B19V.[52] However, estimates of the prevalence of lower levels of B19V DNA vary widely depending on the sensitivity of the method used,[57] with approximately 1% of donations having detectable B19V DNA by sensitive polymerase chain reaction (PCR).[58] The significance of these low levels of B19V DNA in blood samples is unknown.[57]

Mechanism and Routes of Transmission

B19V infections in temperate climates are more common in the late winter, spring, and early summer months.[59] Rates of infection may also increase every 3 to 4 years, as reflected by corresponding increases in the major clinical manifestations of B19V infection, TACs, and EI.[60,61]

B19V DNA has been found in the respiratory secretions of patients at the time of viremia,[62] suggesting that infection is generally spread by a respiratory route of transmission. The virus can be readily transmitted by close contact, and the secondary attack rate has been calculated in various settings; in one study the rate of secondary attack from symptomatic TAC or EI patients to susceptible (IgG-negative) household contacts was approximately 50%.[62] For school outbreaks, serologic studies are generally not available, but 10% to 60% of students may develop a rash consistent with B19V infection.[63,64] The highest secondary attack rates and annual seroconversion rates, even in the absence of known community outbreaks, are for workers in close contact with affected

children, such as daycare providers and school personnel.[65] Nosocomial transmission in hospital situations has been described[66] but is probably infrequent, especially from patients with chronic infection. Nevertheless, patients with TAC or persistent disease should be considered infectious and appropriate precautions taken to limit interaction with other patients and susceptible staff.

The virus can be found in serum, and infection can be transmitted by blood and blood products,[67] including albumin and plasma.[68,69] As described previously, parvoviruses, including B19V, are very heat resistant, and they can withstand the usual thermal treatment aimed at infectious agents in blood products. In addition, solvent-detergent methods, which inactivate only lipid-enveloped viruses, are ineffective. B19V infection has been transmitted by steam-treated, dry-heated, and solvent-detergent-treated factors, although hemophiliacs who received heat-treated factor VIII alone had a lower prevalence of B19V antibody and lower rates of seroconversion than those receiving non–heat-treated factor.[70]

CLINICAL MANIFESTATIONS

Erythema Infectiosum

Manifestations of B19V infection vary, even in the normal host, from asymptomatic or subclinical infection (most people with B19V-specific antibody have no recollection of any specific symptoms) to a biphasic illness with symptoms during the viremic and immune complex–mediated stages of the disease, but EI is the major manifestation. EI was well characterized clinically before the discovery of B19V.[71] This exanthematous rash illness of childhood was probably first described by Robert Willan in 1799 and illustrated in his 1808 textbook. The disease was rediscovered in Germany, where in 1899 Sticker termed it *erythema infectiosum,* and 6 years later Cheinisse classified it as the "fifth rash disease" of the six classic exanthems of childhood.[72] Often the epidemiologic data suggested "a common-source exposure to a highly effective transmitter," and an atypical rubella virus or echovirus was thought to be responsible. However, neither virus could be reproducibly isolated from patients with fifth disease. In 1983, after an outbreak of EI in London, all 31 affected children or adolescents had anti–B19-specific IgM.[73] Similar results were obtained in other epidemics of fifth disease, and B19V is now recognized as the etiologic agent.

Clinical symptoms begin with a nonspecific prodromal illness, which often goes unrecognized; there may be symptoms of fever, coryza, headache, and mild gastrointestinal distress, including nausea and diarrhea. In 2 to 5 days the classic "slapped cheek" rash appears, a fiery red eruption on the cheek, accompanied by relative circumoral pallor (Fig. 147.4). There may be a second-stage rash within a few days and an erythematous maculopapular exanthem on the trunk and limbs; as this eruption fades it produces a typical lacy appearance. There is great variation in the dermatologic symptoms: The classic slapped cheek is much more common in children than adults; the second-stage eruption may vary from a faint, barely perceptible erythema to a florid exanthem; and the rash may be transient or recurrent for weeks. Rarely, other dermatologic presentations are seen: vesicopustular rash,[74] papular-purpuric glove and sock syndrome,[75] other purpuric rashes with or without Koplik spots,[76] and erythema multiforme.[77] Pruritus, especially on the soles of the feet, can be the dominant symptom.[78]

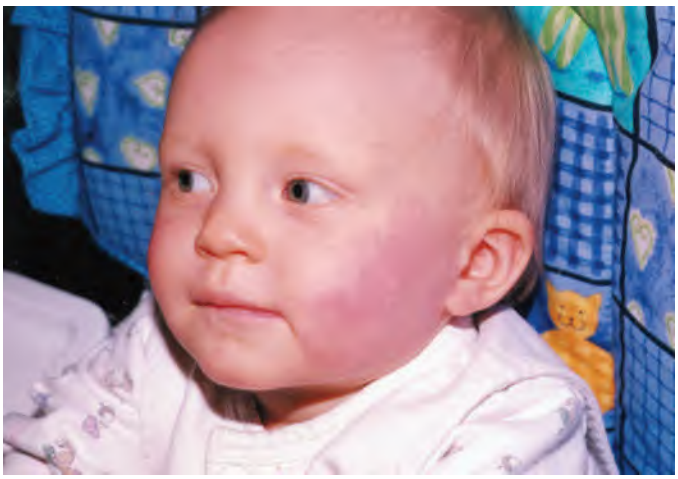

FIG. 147.4 Slapped-cheek appearance of a child with fifth disease.

Arthropathy

Although B19V infection in children is usually mild and of short duration, a large proportion of adults, especially women, suffer arthralgia or frank arthritis, with painful joints often accompanied by swelling and stiffness.[64] The arthralgia is usually symmetrical, with mainly the small joints of hands and feet involved, and generally lasts for 1 to 3 weeks, although it may persist or recur for months or even years. In the absence of a history of rash the symptoms may be mistaken for those of acute rheumatoid arthritis, especially because prolonged symptoms do not correlate with serologic studies, such as the duration of B19V IgM response, or persistent viremia. In addition, B19V infection can be associated with transient rheumatoid factor production.[79] In one large study of patients attending an "early synovitis" clinic in England, 12% had evidence of recent infection with B19V.[80] Three patients would have fulfilled the American Rheumatism Association's diagnostic criteria for definite rheumatoid arthritis. B19V infection should be considered as part of the differential diagnosis in any patient presenting with acute arthritis.

It has been postulated that B19V is involved in the initiation and perpetuation of rheumatoid arthritis leading to joint lesions,[81] but these results have not been reproducible by other groups. In contrast, parvoviral B19V DNA is frequently found in synovial tissue of patients with rheumatoid arthritis, chronic arthropathy, and control subjects. In one carefully performed controlled study, although B19V DNA was indeed detected in synovial tissue of 28% of individuals with chronic arthritis, it was also found in 48% of nonarthropathy controls,[82] indicating that PCR-detectable DNA may persist in synovial tissues for months or years. In addition, in one study with long-term follow-up, none of the 54 patients with B19V-associated arthralgia reported persistence of joint swelling or restricted motion, and no evidence of inflammatory joint disease was found.[83] Therefore it seems unlikely that B19V plays a role in classic erosive rheumatoid arthritis. The association of B19V and juvenile rheumatic disease is more convincing,[84] but whether it is the cause of the disease or one of many potential triggers is less clear.

Transient Aplastic Crisis

TAC, the abrupt cessation of erythropoiesis characterized by reticulocytopenia, absent erythroid precursors in the bone marrow, and precipitous worsening of anemia, was the first clinical illness associated with B19V infection. When stored sera from children admitted to a London hospital were examined for B19V, samples from six Jamaican immigrants with sickle cell disease presenting with aplastic crisis showed evidence of recent infection with B19V (either antigenemia or seroconversion).[85] Retrospective studies of sera from Jamaican patients with sickle cell disease showed that 86% of TACs were associated with recent parvovirus infection.[86]

TAC caused by B19V has now been described in a wide range of patients with underlying hemolytic disorders, including hereditary spherocytosis, thalassemia, red cell enzymopathies such as pyruvate kinase deficiency, and autoimmune hemolytic anemia.[87] TAC can also occur under conditions of erythroid "stress," such as hemorrhage, iron deficiency anemia, and kidney or bone marrow transplantation. Acute anemia has been described in hematologically normal persons,[88] and a drop in red cell count (within the normal range) and reticulocytes was seen in healthy volunteers.[43]

Although suffering from an ultimately self-limiting disease, patients with aplastic crisis can be severely ill. Symptoms may include dyspnea, lassitude, and even confusion related to the worsening anemia. Congestive heart failure, severe bone marrow necrosis,[89] and cerebrovascular complications[90] can develop, and the illness may be fatal. Aplastic crisis can be the first presentation of an underlying hemolytic disease in a well-compensated patient.

Community-acquired aplastic crisis is almost always due to B19V,[91] and B19V infection should be the presumptive diagnosis in any patient

with anemia related to abrupt cessation of erythropoiesis as documented by reduced reticulocytes and bone marrow appearance. In contrast to patients with EI, patients with TAC are often viremic at the time of presentation, with concentrations of virus as high as 10^{15} genome copies/mL (IU/mL); thus the diagnosis is readily made by detection of B19V DNA in the serum. As B19V DNA levels fall in serum, B19V-specific IgM becomes detectable. TAC is easily treated by blood transfusion. After acute infection, immunity is lifelong.

TAC and B19V infection in hematologically normal patients are often associated with changes in other blood lineages, varying degrees of neutropenia,[92] and thrombocytopenia.[93] Some cases of idiopathic thrombocytopenic purpura and Henoch-Schönlein purpura[94] have been reported to follow B19V infection. Transient pancytopenia after parvovirus infection is rare. Although some cases of chronic neutropenia of childhood have also been ascribed to B19V infection,[95] other studies have not confirmed an association.[96]

B19V does not appear to be the cause of true (chronic) aplastic anemia[97] or transient erythroblastopenia of childhood (TEC),[98] the temporary failure of red cell production in normal children. Sporadic cases of TEC with thrombocytopenia with evidence of recent B19V infection have been described, whereas "classic" TEC is associated with *high* platelet counts.

Pure Red Cell Aplasia

Persistent B19V infection that results in PRCA has been reported in a wide variety of immunosuppressed patients, including patients with congenital immunodeficiency, acquired immunodeficiency syndrome (AIDS), and lymphoproliferative disorders and transplant recipients.[99] The stereotypical presentation is with persistent anemia rather than immune-mediated symptoms of rash or arthropathy. Patients have absent or low levels of B19V-specific antibody and persistent or recurrent parvoviremia as detected by B19V DNA in the serum. Bone marrow examination generally reveals the presence of scattered giant pronormoblasts. Administration of immunoglobulin can be beneficial and ameliorative, if not curative.[100]

The prevalence of B19V-induced anemia in human immunodeficiency virus (HIV)-seropositive patients is probably higher than that recognized. In one early study of 50 patients with AIDS, no patients with B19V viremia were identified. In a larger cohort study, B19V DNA was found in only 1 of 191 (0.5%) HIV-seropositive homosexuals. However, B19V DNA was found in 5 of 30 (17%) transfusion-dependent HIV-seropositive homosexuals, and when a hematocrit of less than 20 mL/dL was used as a criterion, 4 of 13 (31%) were positive.[101] In contrast to the earlier studies the marrow morphology need not be suggestive of PRCA, and giant pronormoblasts may not be present.

In less severely immunosuppressed patients (e.g., patients with systemic lupus erythematosus receiving steroid therapy), prolonged anemia after B19V infection has also been described.[102] However, in these patients there was a spontaneous, albeit delayed, development of antibodies, and viremia resolved without therapy. Such patients represent one end of the spectrum of disease manifestations of B19V in patients with a compromised immune system.

Virus-Associated Hemophagocytic Syndrome

Virus-associated hemophagocytic syndrome (VAHS) is characterized by histiocytic hyperplasia, marked hemophagocytosis, and cytopenia in association with a systemic viral illness.[103] In contrast to malignant histiocytosis, VAHS is usually a benign, self-limiting illness in which histiocytic proliferation is reversible. Hemophagocytosis is not uncommon and occurs in the setting of a wide range of infections, not only viral but also bacterial, rickettsial, fungal, and parasitic.[104] However, in many patients there is underlying immunosuppression, usually iatrogenic, so the role of the incriminated pathogen as an etiologic agent or coincidental opportunistic infection remains unclear.

B19V infection has been detected in 15 cases of hemophagocytosis syndrome among children and adults.[105] The majority of patients were previously healthy, but four patients were immunosuppressed by drug therapies. In all but 1 case there was a favorable outcome (one immunosuppressed patient died of fulminant aspergillosis). Further studies are required to determine whether B19V is a major cause of VAHS and what the rate of VAHS is in otherwise uncomplicated B19V infection.

Fetal Infection (Hydrops Fetalis and Miscarriage)

B19V causes 10% to 15% of all cases of nonimmune hydrops fetalis.[106] Nonimmune hydrops fetalis is rare (1 per 3000 births), and in approximately 15% of cases the etiology is unknown. In a study of 63 cases, 8 were due to B19V infection. When pathologic studies have been undertaken, B19-infected fetuses showed evidence of leukoerythroblastic reaction in the liver and large pale cells with eosinophilic inclusion bodies and peripheral condensation or margination of the nuclear chromatin. B19V DNA can be detected by PCR and in situ hybridization, and viral particles by electron microscopy.

Even in the absence of treatment, an adverse fetal outcome is not typical after maternal B19V infection. In a prospective British study of more than 400 women with serologically confirmed B19V during pregnancy, the excess rate of fetal loss was confined to the first 20 weeks of pregnancy and averaged only 9%.[107] No abnormalities were found at birth in the surviving infants, even when there was evidence of intrauterine infection by the presence of B19V IgM in the umbilical cord blood, and there were no long-term sequelae in the 129 children observed for more than 7 years. Similar findings have been found in studies in other countries.[108,109]

No systematic studies have shown evidence for congenital abnormalities after B19V infection,[107,110] although there are case reports of congenital ocular and neurologic abnormalities after maternal B19V infection. Rare cases of congenital anemia after a history of maternal B19V exposure have been reported.[111,112] In these cases the virus load is generally low, and the anemia does not respond to immunoglobulin therapy. The B19V infection may mimic Diamond-Blackfan anemia,[113] and investigation of erythrocyte enzyme activity and ribosomal protein genes may be needed to distinguish the two.[114]

Other Disease Manifestations

B19V infection has been associated with a range of other disease manifestations, including neurologic disease, myocarditis, kidney disease, hepatitis, and vasculitis. However, most of these are case reports or limited PCR-based studies with poorly documented controls. Determining the role of B19V in these diseases is often difficult; the diseases are rare, and B19V may not be the only cause. In addition, with sensitive PCR-based assays, B19V DNA can be detected in many tissues, including bone marrow, synovial, and other tissues from healthy individuals, probably lifelong after infection.[23,115] If the disease is rare, large multicenter trials may be required to substantiate or disprove the causal relationship.

Encephalitis and, more often, aseptic meningitis have been described in serologically confirmed B19V infection[116] and detection of B19V DNA in cerebrospinal fluid. The long-term outcome of infection was generally favorable, and only rarely did long-term sequelae occur. Brachial plexus neuropathy with weakness and sensory loss has also been described in patients with B19V infection,[117] and in one study, 50% of patients with classic fifth disease (confirmed serologically) experienced neurologic symptoms (tingling and numbness in the fingers or toes).[118]

There have been several case reports of myocarditis associated with B19V infection in both children and adults.[119,120] In many of the case reports the diagnosis of B19V as the cause is made simply on the detection of the B19V DNA genome, and given the known persistence of B19V DNA in tissues, this may be erroneous. However, the putative role of B19V in the pathogenesis of myocarditis warrants further investigation, particularly because P antigen is found on fetal myocardial cells, and B19V appears to cause myocarditis in the fetus.[45,46]

Similarly, a number of case reports have described an association of B19V infection and glomerulonephritis in both children and adults.[121]

The role of B19V in both hepatitis and vasculitis remains unclear. Although transient elevation of liver transaminases is not uncommon in B19V infection, frank hepatitis associated with B19V infection has rarely been reported.[122] B19V has been suggested as a possible causative agent of fulminant liver failure and associated aplastic anemia on the

basis of PCR studies.[123] However, the detection of B19V DNA in control liver tissue is not uncommon, and with appropriate controls we have been unable to confirm this putative relationship.[26,124]

Several case reports have described positive B19V serology in patients with vasculitis or polyarteritis nodosum, systemic necrotizing vasculitis, and Kawasaki disease, a multisystem vasculitis of early childhood. However, other studies have failed to confirm a relationship between B19V and vasculitis[125] or Kawasaki disease.[126]

IMMUNE RESPONSE

Both virus-specific IgM and IgG antibodies are made after experimental[43] and natural B19V infection (see Fig. 147.3). After intranasal inoculation of volunteers, virus can be detected first at days 5 to 6, and levels peak at days 8 to 9. IgM antibody to virus appears about 10 to 12 days after experimental inoculation, and IgG antibody appears at about 2 weeks (see Fig. 147.3). The time course is similar in natural infections. In patients with TAC, 10^9 to 10^{14} genome copies per milliliter of viral DNA may circulate. IgM antibody may be present in patients with TAC at the time of reticulocyte nadir and during the subsequent 10 days; IgG usually appears during the period of hematopoietic recovery. High-titer viremia is not detectable in patients with clinical fifth disease (the manifestations are secondary to immune complex formation).

IgM antibody may be found in serum samples for several months after exposure.[127] IgG persists for life, and levels rise with reexposure.[43] Measurable IgA antibodies specific to B19V may play a role in protection against infection by the nasopharyngeal route.[128]

In immunocompetent individuals the early antibody response is to the major capsid protein VP2, but as the immune response matures, reactivity to the minor capsid protein VP1 dominates. Sera from patients with persistent B19V infection typically contain antibody to VP2 but not to VP1.[129] The importance of an immune response to VP1 for protective immunity has been confirmed in animal experiments using recombinant capsids. Rabbits immunized with capsids containing only VP2 produced a strong antibody response, as measured by enzyme-linked immunosorbent assay (ELISA), but the sera had low neutralization titers. In contrast, rabbits immunized with capsids containing VP1 produced antibody with neutralizing titers comparable with those produced in humans after acute B19V infection.[130] In addition, the importance of the humoral arm of the immune response is shown by recovery from infection with the appearance of circulating specific antivirus antibody, and administration of commercial immunoglobulins can cure or ameliorate persistent parvovirus infection in immunodeficient patients.

The role of the cellular immune response in limiting B19V infection has been studied less intensively. Using a combination of recombinant capsids and peptides from the NS and capsid proteins, it is now clear that B19V infection induces profound $CD8^{+}$[131] and $CD4^{+}$[132–134] responses, both of which are required for viral clearance.

Persistent B19V infection is the result of failure to produce effective neutralizing antibodies by the immunocompromised host. Perhaps because of the limited numbers of epitopes presented to the immune system by B19V, the congenital immunodeficiency states associated with persistent infection may be clinically subtle, with susceptibility largely restricted to parvovirus, although multiple immune system defects are apparent when direct testing of T- and B-cell function is performed.

DIAGNOSIS

There is no suitable method for virus isolation from clinical specimens, and the detection of virus relies on the detection of B19V DNA by quantitative PCR. In immunocompetent individuals, B19V DNA is detectable at very high titer (>10^9 IU/mL) for 2 to 4 days (see Fig. 147.3) and then drops to between 10^5 and 10^6 IU/mL as the immune response develops. The development of the antibody response correlates with the appearance of the rash, and therefore the diagnosis of acute infection due to B19V infection is generally based on IgM assays, ideally performed by the capture technique.[135] In an ELISA, antibody can be detected in greater than 90% of cases by the third day of TAC or at the time of rash in EI. IgM antibody remains detectable for 2 to 3 months after infection.

B19V IgG can be detected by capture assay or indirect assay. IgG is usually present by the seventh day of illness and is probably present for life thereafter. Because greater than 50% of the population has IgG antibody to B19V infection, this test is not helpful for the diagnosis of acute infection.

Immunocompromised or immunodeficient patients with hematologic disease due to chronic infection typically do not mount an immune response to the virus, and testing for B19V DNA is necessary to document recent infection. However, this needs to be done by quantitative PCR because even in immunocompetent persons, low levels of B19V DNA may be detectable by PCR for more than 4 months in serum after acute infection[136,137] and for years in bone marrow, synovium, liver, heart, and other tissues.[23,115] As noted earlier in this chapter, detection of B19V DNA in tissues does not prove that the disease is due to B19V infection. In general, the diagnosis of acute or chronic infection can be made on the basis of quantitative (real-time) PCR in combination with serologic assays for B19-specific IgG or IgM, or both.[138]

Investigation of B19V fetal or congenital infection should be accompanied by serologic studies of the maternal serum. At the time of fetal infection the mother should have evidence of recent B19V infection with detectable IgG and possibly IgM. If the IgM titer is low or absent, recent infection can be documented using IgG avidity studies or measuring B19V DNA levels.[139] Fetal infection can be confirmed by amniotic fluid sampling, by fetal blood sampling, or from postmortem tissue.

THERAPY

In the overwhelming majority of children and adults, B19V infection is a benign and self-limiting infection that results in lifelong immunity and requires no treatment other than symptomatic relief. Patients with arthralgia and arthritis usually respond to nonsteroidal antiinflammatory drugs, although in some patients symptoms can persist for months and even years.[80] In patients with hematologic disease or persistent infection, specific treatment may be necessary. Cidofovir, an acyclic nucleoside phosphonate that is approved for treatment of herpes simplex virus and cytomegalovirus infections, has been shown to have activity against B19V in vitro, but in vivo studies have not been carried out.[140]

Immunocompetent patients with TAC have a self-limiting illness, and typical TAC is readily treated by blood transfusion and supportive therapy alone. In one study of sickle cell patients with aplastic crisis, 87% required blood transfusions, and 61% required hospitalization for their symptoms. One death occurred before transfusion could be given,[141] which underscores the importance of prompt medical intervention.

In immunosuppressed patients with documented, persistent B19V infection, temporary cessation of immunosuppression may be sufficient to allow the host to mount an immune response and resolve the B19V infection, and no additional treatment is required. In cases in which cessation of immunosuppression is not feasible or is ineffective, administration of immunoglobulin can be beneficial.[47,100,142] The usual regimen is intravenous IgG at a dose of 0.4 g/kg for 5 days. Patients often respond with a marked reduction in the level of B19V viremia, reticulocytosis, and resolution of the anemia within 1 to 2 weeks of treatment. However, monitoring for relapse is important, by observation of the reticulocyte counts and quantitative assays for B19V DNA when indicated. If relapse occurs less than 6 months after the initial treatment, especially in HIV-positive patients, an empirical maintenance treatment with a single-day infusion of 0.4 g/kg IgG every 4 weeks may control the B19V viremia.

The role of intrauterine blood transfusions in the treatment of hydrops fetalis related to maternal B19V has been shown to be beneficial.[143,144] However, intrauterine blood transfusions have risks. B19V-associated hydrops is known to resolve spontaneously, and the fetus can be normal at delivery. In addition, there remains the theoretical risk that treatment may be confounded by an increased incidence of antibody-enhanced infection and damage, especially to myocardial cells and the immune system.[111]

PREVENTION AND VACCINATION

The only measures currently available to prevent B19V infection are those designed to interrupt virus transmission. However, because patients

are viremic and infectious before the symptoms of EI, isolation of patients with fifth disease is not rational. Patients with TAC and PRCA are both viremic and infectious and should be appropriately separated from high-risk contacts. The Centers for Disease Control and Prevention recommend that patients with TAC have droplet isolation precautions for 7 days, and for patients with chronic infection, isolation should be continued for the duration of their hospitalization.[145]

The humoral immune response plays the major role in the normal immune response to parvovirus. Although antibodies appear protective in both passive and active immunizations, insufficient data are available to assess the efficacy of immunoprophylaxis.[146,147]

Prospects for vaccination are favorable, with a B19V empty capsid vaccine currently under development. The presence of VP1 protein in the capsid immunogen appears critical for the production of antibodies that neutralize virus activity in vitro, and capsids with supranormal VP1 content are even more efficient in inducing neutralizing activity in immunized animals. Although phase I trials of a VP1-enhanced baculovirus-produced B19V vaccine looked promising,[148] the most recent study has been halted due to unexplained cutaneous reactions in 3 of the 43 patients enrolled, and further studies are on hold.[149] A candidate virus-like particle (VLP) vaccine has been generated in yeast, consisting of both VP1 and VP2, which is phospholipase negative and can be highly purified, with the intent to reduce reactogenicity.[150] Even with an effective and safe vaccine, the target populations for such a vaccine remain to be determined. Should only those at high risk for severe or life-threatening disease, such as sickle cell patients, be protected? Or, in view of the wide variety of disease manifestations affecting all strata of the population, should a universal vaccine policy be pursued?

OTHER HUMAN PARVOVIRUSES

Human Dependoparvoviruses

At least nine different primate dependoparvoviruses have been described,[151] and AAVs 1, 2, 3, 8, and 9 are common agents of human infections.[151] Although AAV DNA has been detected in a wide range of tissues, including some fetal abortion tissues, none of the dependoparvoviruses have been definitively linked with disease in either humans or animals. This lack of pathogenicity, plus the ability of the genome to infect both dividing and nondividing cells and to assess transgenes for extended periods with or without integration into the human genome, has made the dependoparvoviruses popular choices to modify and use as gene therapy vectors in a number of different clinical settings, including treatment for monogeneic diseases and against infectious diseases.[152,153]

Human Bocaparvoviruses

Bocaparvoviruses are parvoviruses that infect the respiratory and gastrointestinal tracts of young animals and humans. In 2005 sequences of the first human bocaparvovirus (HBoV1) were identified in respiratory samples of Swedish children with lower respiratory tract infections using a molecular screening method.[154] The nucleotide sequence of HboV1 was determined and shown to have the same genomic organization as other members of the bocaparvovirus genus, with a third middle open reading frame. However, the sequence was significantly different from the other known animal bocaparvoviruses at the time. Subsequently, related viruses—human bocaparvoviruses 2 to 4 (HboV2, HboV3, and HboV4)—have been detected in fecal samples.[155–157]

Human bocaparvoviruses have a worldwide distribution and have been identified in all countries that have looked for them. HboV1 is predominantly found in respiratory secretions and is found in 2% to 20% of respiratory secretions in prevalence studies primarily from children with acute respiratory disease.[158] Although HboV1 DNA can be detected in respiratory secretions throughout the year, primary infection is predominantly in the winter and spring months, as for many other respiratory infections. HboV-specific antibodies can now be detected using binding assays with recombinant viral capsid proteins,[159] and seroepidemiology studies indicate that HboVs are a common infection of early childhood, with most acquiring antibodies in the first 4 years of life.[160] In contrast to HboV1, HboV2 to HboV4 are predominantly found in fecal samples of both children and adults. Seroprevalence studies in adults indicate that 30% to 50% of adults have antibodies to HboV2, 8% to 38% to HboV3, and 1% to 4% to HboV4.[158]

Despite the observation that HboV1 is often found with other pathogens, there is increasing evidence that the virus itself is pathogenic and especially associated with wheezing and respiratory disease in young children.[161] Several studies have reported that HboV1 is more frequent in patients with respiratory tract symptoms than in asymptomatic individuals.[162–165] A variety of respiratory diseases have been implicated in HboV1 infection, including lower and upper respiratory tract illnesses,[159,166] primarily in young children. HboV1 infections in otherwise normal adults with respiratory illnesses appear to be uncommon but have been reported mainly from immunocompromised individuals.[165–168]

The association of HboV1 infection with respiratory illness is confounded not only by the frequent coinfection with other respiratory viruses but also by the persistence of detectable HboV1 DNA in respiratory and fecal samples and the long period of HboV1 shedding after infection. It is now recognized that diagnosis of HBoV1 infection should not be based on detection of HboV1 DNA alone in respiratory or fecal samples, but on detection of high-titer viral load ($>10^4$ genome copies/mL or respiratory secretion), viral DNA in serum, serologic evidence of recent infection, or a combination of these.[155,169] Without this combination of data the etiologic significance of detection of bocavirus DNA remains uncertain,

Currently there is no specific treatment for any of the bocaparvovirus infections.

Parv4

Using similar methods to those used to identify HBoV1, a fourth group of parvoviruses, human parvovirus 4 (Parv4),[170] was also found in 2005, with three different genotypes of Parv4 identified to date.[171] The virus has not been grown in culture, but studies of the viral sequence and transcription map[172] suggest that Parv4 does not group with any of the other parvovirus genera and has now been placed in the new parvovirus genus, *Tetraparvovirus*. The viral sequences are commonly found in pooled serum samples[173] and in bone marrow and lymphoid tissue of injection drug users and hemophiliacs.[174] Seroprevalence studies indicate that although antibody is rarely found in blood donors in Europe,[175,176] it is found in those who share needles or receive blood products[177] and at significantly higher rates in parts of Africa.[178,179] It is still unclear which is the main route of transmission, with data suggesting parenterally in Europe, but by fecal-oral routes or even respiratory routes in Africa.[180] Seroconversions to Parv4 have rarely been documented, but primary infection may be associated with a rash.[181] Further information about the pathogenicity of this virus is necessary.

Human Protoparvoviruses

Metagenomic next-generation sequencing has also been used to identify three groups of human protoparvoviruses in human fecal samples: BuVs in 2012, TuV in 2014, and CuV in 2016.[2] Limited information is known about any of these viruses, although BuV has only been found in samples from patients with diarrhea, suggesting that it may be a cause of gastroenteritis. CuV has been found in skin biopsies and fecal material, but the significance of this is unknown.

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148 Orthoreoviruses and Orbiviruses

Kenneth L. Tyler

SHORT VIEW SUMMARY

Definition
- Reoviruses are linear double-stranded RNA viruses with broad host ranges.
- The term *reovirus* is an acronym for respiratory enteric orphan virus, which emphasizes the anatomic site from which these viruses were initially isolated.

Epidemiology
- Infection of humans is common but is rarely associated with significant disease.
- Asymptomatic infection is common; symptomatic infection usually consists of mild, self-limited upper respiratory tract and gastrointestinal illness.
- Reoviruses have been identified as the causative agent in rare human cases of meningitis, encephalitis, pneumonia, and myocarditis and are potentially associated with biliary atresia and choledochal cysts.
- Orthoreoviruses are USED in human clinical trials as oncolytic agents.

Microbiology
- Five genera of the Reoviridae have been etiologically linked with diseases of humans: *Orthoreovirus, Orbivirus, Rotavirus, Coltivirus,* and *Seadornavirus*.
- Double-stranded RNA is organized into 10 segments (orthoreoviruses and orbiviruses), which are capable of reassortment and resultant generation of novel viruses.

Diagnosis
- Laboratory diagnosis can be made: (1) serologically by demonstrating a fourfold rise in virus-specific antibody titer between acute and convalescent serum, (2) by virus isolation from serum, stool, respiratory secretions, or cerebrospinal fluid (CSF), (3) by nucleic acid amplification from body fluids or tissues using reverse-transcriptase polymerase chain reaction (RT-PCR) techniques, or (4) by detecting viral antigens in tissues from biopsy or autopsy specimens with immunocytochemistry using virus-specific antibodies.

Therapy
- No specific therapy is available.

Prevention
- No specific preventative measures are recommended; reoviruses are ubiquitous.

The Reoviridae family of viruses consists of 15 genera, whose members have a widely varied host range, including plants and invertebrate (insects, crustaceans) and vertebrate (mammalian, reptilian, avian) animals. Five genera have been etiologically linked with diseases of humans: *Orthoreovirus, Orbivirus, Rotavirus, Coltivirus,* and *Seadornavirus*; the first two are covered in this chapter and the others in additional chapters. The genome of all Reoviridae consists of linear double-stranded RNA surrounded by a nonenveloped icosahedral capsid 60 to 80 nm in diameter. Genomic material is organized into 10 to 12 segments, which are capable of reassortment and resultant generation of novel viruses.[1] In this chapter, clinically relevant information is presented pertaining to human infection due to orthoreoviruses and orbiviruses. Rotaviruses, coltiviruses, and seadornaviruses are discussed in Chapters 149 and 150.

ORTHOREOVIRUSES

Background and Epidemiology

Reoviruses were first discovered in the 1950s after isolation from human enteric specimens, and they were subsequently documented to infect a wide range of hosts including mammals, birds, reptiles and fish. Mammalian *Orthoreovirus* serotypes 1, 2, and 3 are found ubiquitously in the environment, and their sources include stagnant and river water and untreated sewage. The term *reovirus* is an acronym for respiratory enteric orphan virus, which emphasizes the anatomic site from which these viruses were initially isolated and the fact that infection of humans, although common, is only rarely associated with significant disease. Human infection primarily results in either asymptomatic infection or mild, self-limited symptoms such as upper respiratory tract illness and gastroenteritis.[2,3] Rarely, reoviruses have been identified as the causative agent in human cases of meningitis, encephalitis, pneumonia, and myocarditis and are potentially associated with biliary atresia and choledochal cysts (discussed later).[2–4] Serologic studies of reovirus prevalence have documented steady increases from infancy (5%–10% seropositivity at 1 year of age; 30% at 2 years of age; 50% at 5 years of age) through adulthood (50% at 20–30 years of age; >80% by 60 years of age),[4,5] reflecting immunity acquired as a consequence of natural infection. Despite this, it has been difficult to definitively correlate seroconversion and primary acute infection with specific diseases. Natural transmission likely occurs by the fecal-oral and airborne routes in humans. Reovirus infection has been used as an experimental animal model system to study viral CNS infections, myocarditis, and hepatobiliary and respiratory diseases.[6]

Clinical Disease

Respiratory Tract Manifestations

Mild upper respiratory tract illness consisting primarily of rhinorrhea and pharyngitis accompanied by low-grade fever, headache, and malaise (35%–80%), with or without mild diarrhea (15%–65%), has been described in children during outbreaks and was produced in experimental reovirus infections of adult volunteers.[7,8] In children, a maculopapular and very rarely a vesicular exanthem has been described,[4] as have coryza and otitis media. Rare reports of lower respiratory tract disease have included interstitial or confluent pneumonia, including fatal cases.[9,10] A novel reassortant reovirus designated as BYD1 strain was isolated in 2003 from five patients with severe acute respiratory syndrome (SARS) who were coinfected with SARS-associated coronavirus. Subsequent characterization in animal experimental models has suggested a possible copathogenic role for this virus in SARS.[11,12]

At least a dozen orthoreoviruses have been isolated from bats (pteropine reoviruses) beginning (1970) with Nelson Bay virus, which serves as the prototypical species. Two closely related pteropine orthoreoviruses (Melaka and Kampar viruses) were identified in 2006 and linked with acute upper respiratory disease in six adult and pediatric Malaysian patients.[13,14] Both viruses are presumably of bat origin with transmission to humans by exposure to bat droppings or contaminated fruits. Serologic studies have suggested that pteropine orthoreovirus infection is ubiquitous in Southeast Asia, and possibly other areas, and that bats can serve as reservoirs for transmission of infection to humans.[15] Index adult cases of pteropine-derived orthoreovirus infection occurred in patients with high fever, chills and rigors, sore throat, headache, and myalgia[13,14]. Human-to-human transmission has been substantiated through serologic analysis of secondary cases, including children. The human seroprevalence rate is unknown, but one study from Vietnam found that 4% of adult sera contained immunoglobulin G (IgG) antibodies against pteropine orthoreovirus, detected with enzyme-linked immunosorbent assay (ELISA); half of these individuals also had neutralizing-antibodies.[16] Other studies from Southeast Asia have suggested higher seroprevalence rates, with results up to 17% among outpatients presenting with upper respiratory infections.[17,18] At least six cases of acute respiratory tract infection, similar to those described earlier, have been associated with Nelson Bay virus, the prototypical pteropine orthoreovirus.[19] Isolated cases of human disease linked to other pteropine orthoreovirus isolates (Hong Kong HK23629/07, HK46886/09, and HK50842/10; Sikamat virus) have been reported.[17,20,21]

Gastrointestinal and Hepatobiliary Manifestations

A long-term study of children with diarrhea implicated reoviruses in only 0.1% of cases, and those occurred mainly in infants younger than 1 year.[2] A role for reovirus infection in the pathogenesis of extrahepatic biliary atresia and choledochal cysts has long been proposed based on similarities between pathologic changes observed in pediatric patients with these diseases and reovirus-infected mice.[22] However, results from serology-based and more recent molecular-based studies are conflicting.[22,23] In one study, reovirus RNA was detected in hepatic or biliary tissues from 55% of patients with extrahepatic biliary atresia and 78% of patients with choledochal cysts, compared with 21% of patients with other hepatobiliary diseases and 12% of autopsy cases.[22] However, a subsequent study failed to detect reovirus genome in hepatobiliary tissues taken from 26 patients with extrahepatic biliary atresia and 28 patients with congenital dilation of the bile duct.[23] No convincing data exist to support an etiologic role for reovirus infection in the setting of idiopathic cholestatic liver diseases in adults.

Central Nervous System Manifestations

Viruses belonging to orthoreovirus serotypes 1, 2, and 3 have been isolated from cerebrospinal fluid (CSF) of infants with meningitis, systemic illness, or both. An orthoreovirus serotype 1 was isolated from a previously healthy 3-month-old child with symptoms of meningitis, diarrhea, vomiting, and fever.[24] Orthoreovirus serotype 2 (subsequently identified as a new mammalian orthoreovirus type 2 Winnipeg) was isolated from the CSF of an 8-week-old child with active varicella-zoster virus infection, *Escherichia coli* sepsis, intermittent fever, diarrhea, and feeding intolerance.[25,26] A novel orthoreovirus belonging to serotype 3 reovirus (T3C/96) was isolated from a 6-week-old child with meningitis and was subsequently shown to be capable of producing lethal encephalitis in neonatal mice.[27] A novel orthoreovirus strain belonging to serotype 2 (MRV2Tou05), was implicated in two cases of acute necrotizing encephalopathy (ANE) in children from the same family.[28] These reports illustrate the rare but possible neuroinvasive potential of reoviruses in the human host.

Reovirus as an Oncolytic Agent

Orthoreoviruses induce apoptosis in multiple cell types.[6] They replicate preferentially in cells with activated *ras* genes or ras signaling pathways, which is the case in as many as 60% to 80% of human malignancies and 90% of metastatic cancers.[29] A number of preclinical studies have demonstrated the potential application of serotype 3 orthoreovirus as an anticancer oncolytic agent.[30] Multiple tumor types have been shown to be susceptible to orthoreovirus infection and oncolysis in vitro, including breast, ovarian, brain (glioma, medulloblastoma), colon, bladder, pancreatic, prostate, and lung cancers and melanoma; childhood sarcoma; and head and neck tumors.[31,32] Human clinical trials with proprietary formulations of mammalian reovirus serotype 3 Dearing (pelareorep [Reolysin]; Oncolytics Biotech, Calgary, Alberta, Canada) began in 2002. Patients have been treated in phase I, II, and III trials with pelareorep (Reolysin) intratumorally or intravenously, alone or in combination with other chemotherapeutic agents or radiation therapy.[33,34] The most recently published findings have included lack of efficacy of adjunctive orthoreovirus therapy to chemotherapy alone in trials involving recurrent ovarian, tubal, or peritoneal cancer[35] and metastatic pancreatic cancer,[36] but a possible added benefit against metastatic or recurrent small cell lung cancer[37] and potential efficacy in advanced malignant melanoma.[38] A phase II study in metastatic breast cancer suggested that addition of oncolytic reovirus (pelareorep) to chemotherapy did not enhance progression-free survival or recurrence rate but did increase overall survival from 10.4 months to 17.4 months, although the result did not reach statistical significance ($P = .1$).[39] In May 2017, the FDA granted pelareorep "fast track" designation for studies in treatment of metastatic breast cancer.

Although reovirus-based oncotherapy primarily targets cancer cells through direct killing by apoptosis (oncolysis), additional immune-based mechanisms of oncolysis including enhancement of antibody-dependent cellular cytoxicity have also been reported.[40,41] Synergistic antitumor effects of reovirus in combination with radiation or chemotherapy have been demonstrated.[41,42] In general, efficacy occurs in tumors with activated Ras signaling pathways. Characterization of immune responses to intravenous Reolysin have been reported[43]; in most clinical trials to date, the antireovirus immune response has been observed to correlate with decreased efficacy. Investigation into the use of reovirus as an immune adjuvant is underway with the goal of redirecting immune responses to target tumors.[42]

ORBIVIRUSES

Background and Epidemiology

The genus *Orbivirus* contains more than 130 subspecies classified within 19 species and/or antigenic serogroups, infecting a broad range of arthropod and vertebrate hosts. Orbiviruses are named based on their characteristic doughnut-shaped capsomers. The bulk of disease due to orbiviruses occurs in nonhuman vertebrates; the most frequently identified are bluetongue virus (sheep, cattle, goats, and wild ungulates),[44] African horse sickness virus (horses, donkeys, and dogs), and epizootic hemorrhagic disease virus (deer).[45] Disease in humans has been reported infrequently (fewer than 100 cases reported in the literature worldwide). Only one primary orbivirus-linked human disease, Oklahoma tick fever, occurs in the continental United States. This syndrome is associated with elevated antibody titers against Sixgun City and Lipovnik viruses, suggesting a likely causal association with these or a closely orbivirus species. Humans typically serve as an incidental host during the maintenance cycle of vector-borne transmission between nonhuman vertebrate hosts, and human-to-human orbivirus transmission has not been reported. Vectors for disease include mosquitoes, midges, gnats, sand flies, and ticks.[44–47]

Clinical Disease

Only four orbivirus serogroups have been linked to disease in humans; these are the viruses belonging to the Kemerovo antigenic complex, including Kemerovo, Lipovnik, and Tribec viruses (Russia and Eastern Europe; Oklahoma tick fever virus in the United States), Orungo virus (sub-Saharan Africa), Lebombo virus (South Africa and Nigeria), and Changuinola virus (Central America).[45] The spectrum of reported human disease includes neurologic infection (encephalitis, meningitis, meningoencephalitis, polyradiculitis) and acute febrile illnesses. Many orbiviruses preferentially infect vascular endothelial cells; thus clinical and laboratory manifestations can mimic those seen in the setting of rickettsial illnesses. No deaths have been reported due to human orbivirus infection, and patients generally recover without long-term sequelae of infection. All age groups may be infected; however, the pediatric population is

overrepresented in seroprevalence studies. In animals, orbivirus infection has been linked to congenital abnormalities such as hydranencephaly, arthrogryposis, and deafness, but these syndromes or illnesses have not yet been reported in humans.[45]

Clinical Manifestations of Specific Agents

Kemerovo Virus Antigenic Complex

Viruses of the Kemerovo complex (Kemerovo, Tribec, and Lipovnik) are transmitted by *Ixodes* spp. ticks in Russia and Eastern Europe and were first isolated in 1963 from ticks and patients with meningitis and meningoencephalitis. Meningoencephalitis and polyradiculitis have been linked to Lipovnik virus in the present-day Czech Republic. Seroprevalence studies in healthy residents of the former Czechoslovakia indicate up to 18% seropositivity; additional serologic evaluation of patients from Central Europe with tick-borne encephalitis virus infection and neurologic symptoms demonstrated the presence of concurrent Lipovnik virus antibodies in more than 50% of patients.[48]

Oklahoma Tick Fever

In the United States, cases of acute febrile illness, subsequently designated as Oklahoma tick fever, have been reported in Oklahoma and Texas and attributed to orbivirus infection, likely by a Kemerovo serogroup–related virus. Clinical features of infected patients included myalgia, vomiting, and severe abdominal pain. Laboratory features included transient leukopenia, thrombocytopenia, and anemia, suggesting possible rickettsial disease; however, serologic analysis for Rocky Mountain spotted fever, Colorado tick fever, and Powassan virus was negative.[44] Diagnosis was based on positive serology for Kemerovo group–related orbiviruses (Sixgun City and Lipovnik viruses). Viremia was not present in these patients. Transmission of Kemerovo-related viruses in rabbit and large animal populations has been documented in states in the Midwest, but no human cases have been reported to date.

Orungo Virus

Orungo virus is transmitted primarily by *Aedes* species but also by *Culex* and *Anopheles* species mosquitoes in regions of sub-Saharan Africa. Seroprevalence rates in that region are as high as 24% to 34%.[49] Acute febrile illness, including fever, headache, and myalgia, has been reported, as has one case of encephalitis in a child with convulsions and flaccid paralysis.[50,51] Coinfection with yellow fever virus has been documented.[52]

Lebombo Virus

Transmission of Lebombo virus occurs from *Aedes* and *Mansonia* species mosquitoes in South Africa and Nigeria. A case of nonspecific acute febrile illness has been reported in a Nigerian child.[50]

Changuinola Virus

Acute febrile illness due to Changuinola virus has been reported in a single human case from Panama. Seroprevalence studies indicate high rates of seropositivity in parts of South America, but the infection-to-disease ratio is unknown. Transmission may occur via phlebotomine flies.

African Horse Sickness Virus

Naturally occurring infection of humans with African horse sickness virus has not been reported. However, four workers in a South African veterinary office were infected in 1989 with accidentally aerosolized freeze-dried virus present in a vaccine containing attenuated viral strains. Illness was severe in all exposed workers; three developed frontotemporal encephalitis, and all four developed uveochorioretinitis. Diagnosis was confirmed serologically.[53]

Diagnosis

Laboratory diagnosis of orbivirus infection is made by (1) a fourfold rise in acute and convalescent serum antibody response as detected by complement fixation, enzyme immunoassay, or neutralization; or (2) virus isolation from serum or CSF by inoculation of suckling mice or cell cultures (Vero or BHK021). Virus-specific immunoglobulin M (IgM) testing is available at reference laboratories, including the Centers for Disease Control and Prevention and the US Army Medical Research Institute of Infectious Diseases. A variety of reverse-transcriptase polymerase chain reaction (RT-PCR) nucleic acid amplification techniques have been developed for research purposes and can be used for identification and molecular typing of veterinary pathogens including bluetongue viruses and epizootic hemorrhagic disease viruses. A consensus orbivirus RT-PCR using primers specific for the viral RNA-dependent RNA polymerase is also available as a research tool.[54] No specific therapy is available.

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The complete reference list is available online at Expert Consult.

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149 Coltiviruses (Colorado Tick Fever Virus) and Seadornaviruses

Daniel M. Pastula and Kenneth L. Tyler

SHORT VIEW SUMMARY

Overview and Virology

- *Coltivirus* and *Seadornavirus* are two of five genera of the Reoviridae virus family that have been documented to cause human disease.
- Coltiviruses and seadornaviruses are nonenveloped double-stranded RNA viruses with a segmented genome (12 gene segments enclosed within two concentric capsid shells).

Epidemiology

- Coltiviruses associated with human disease include Colorado tick fever virus (CTFV; North America), Salmon River virus (unclear if a distinct virus or just a serotype of CTFV; North America), and possibly Eyach virus (Europe).
- The only seadornavirus implicated in human disease to date is Banna virus (southeast Asia and China). Several other novel seadornaviruses have also been identified, though none are known to cause human disease.
- Ticks are thought to be the principal vectors of coltiviruses; mosquitoes are thought to be the principal vectors of seadornaviruses.
- CTFV is endemic to high-elevation (>4000 feet above sea level) western North America, where its principal tick vector—*Dermacentor andersoni,* or the Rocky Mountain wood tick—is found.

Clinical and Laboratory Features

- CTFV infection can cause a febrile illness with chills, headache, myalgias, and/or fatigue. Sore throat, nausea, vomiting, abdominal pain, arthralgia, neck pain or stiffness, and/or rash (either maculopapular or petechial) can also occur.
- Complications of CTFV disease can rarely include meningitis, encephalitis, disseminated intravascular coagulation, myocarditis, pericarditis, pneumonia, hepatitis, and epididymo-orchitis.
- Leukopenia is common in CTFV disease, and thrombocytopenia can occur. Anemia is rare despite CTFV infecting erythrocyte progenitor and red blood cells. Cerebrospinal fluid analysis may show a lymphocytic pleocytosis with mildly elevated protein and near-normal glucose.
- Eyach virus antibodies have been found in patients with polyradiculoneuritis and meningoencephalitis in Europe, but it is unclear whether Eyach virus itself was the cause.
- Banna virus disease can include fever, myalgia, arthralgia, and/or encephalitis.

Diagnosis

- CTFV should be considered in anyone presenting with a febrile illness following tick exposure in high-elevation western North America.
- CTFV nucleic acid can often be detected in the serum for 2 weeks after symptom onset by reverse-transcriptase polymerase chain reaction (RT-PCR).
- CTFV antibody development is often delayed for 2 to 3 weeks; CTFV-specific immunoglobulin M and confirmatory neutralizing antibody tests are available.
- Contact your state or local health department for assistance with CTFV testing.
- Eyach virus– and Banna virus–specific RT-PCR and antibody tests have been developed.

Therapy and Prevention

- No specific therapy is available for CTFV or Banna virus disease.
- Tick-bite and mosquito-bite prevention strategies are important.
- CTFV-infected patients should not donate blood or bone marrow for at least 6 months given the virus's persistence in red blood cells.

Coltivirus and *Seadornavirus* are two of five genera of the Reoviridae virus family that have been documented to cause human disease; *Orthoreovirus, Orbivirus,* and *Rotavirus* genera are discussed in Chapters 148 and 150. Like all Reoviridae, coltiviruses and seadornaviruses are nonenveloped double-stranded RNA viruses with a segmented genome (both have 12 gene segments enclosed within two concentric capsid shells). Coltiviruses were classified within the *Orbivirus* genus until 1991, at which time their distinct molecular identity was established and a unique genus was proposed. Coltiviruses were initially subclassified into the tick-borne subgroup A (North American and European distribution) and mosquito-borne subgroup B (Asian distribution) viruses. In the year 2000, subgroup B coltiviruses were reclassified into the newly designated genus *Seadornavirus* (an acronym for Southeast Asian dodeca RNA virus) based on sequence data and antigenic properties.[1–5]

Coltiviruses associated with human disease include Colorado tick fever virus (CTFV; North America), Salmon River virus (unclear if a distinct virus or just a serotype of CTFV; North America), and possibly Eyach virus (Europe).[5–11] The only seadornavirus implicated in human disease to date is Banna virus (southeast Asia and China).[3,5,12–16] Other novel seadornaviruses such as Liao Ning, Kadiporo, Mangshi, and Balaton viruses have recently been identified, but it is not clear whether they cause human disease.[3,5,17–19]

COLTIVIRUSES

Colorado Tick Fever Virus

Epidemiology

CTFV is endemic to high-elevation (>4000 feet above sea level) western North America, where its principal tick vector—*Dermacentor andersoni,* or the Rocky Mountain wood tick—is found (Fig. 149.1). CTFV is maintained in an epizootic cycle between *D. andersoni* and small, high-elevation mammals such as porcupines, squirrels, chipmunks, and mice.[6,20–23] People are typically infected after being bitten by a CTFV-infected tick; approximately 90% of CTFV disease cases in a recent epidemiologic review reported tick exposure prior to illness onset.[24] Hikers, campers, forestry workers, ranchers, and outdoor laborers in endemic areas are thought to be at particular risk. Rarely, CTFV transmission has also been documented by blood transfusion and laboratory exposure.[25,26]

Because CTFV disease has not been reportable on a national level, exact incidences across states are difficult to calculate, and CTFV disease cases may be underrecognized and underreported.[21,24,27] Two recent epidemiologic reviews suggest that Wyoming currently has the highest incidence of reported CTFV disease cases (3.4–7.2 cases per million people per year), followed by Montana (1.5–3.4) and Utah (0.5–1.4).[21,24] Colorado had the most reported CTFV disease cases

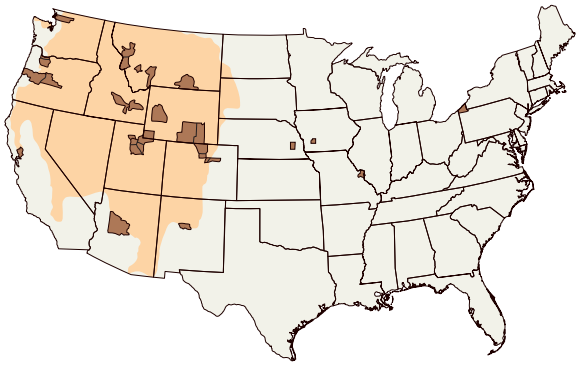

FIG. 149.1 Approximate geographic distribution of *Dermacentor andersoni* ticks and counties of residence for confirmed and probable Colorado tick fever virus (CTFV) disease cases, United States, 2002–2012. *All cases were acquired in states where local transmission of CTFV has been reported previously. Two additional cases were reported from Colorado with unknown county of residence. **Derived from James AM, Freier JE, Keirans JE, et al. Distribution, seasonality, and hosts of the Rocky Mountain wood tick in the United States. *J Med Entomol.* 2006;43:17–24. *(Courtesy Centers for Disease Control and Prevention. Colorado tick fever: statistics and maps.* https://www.cdc.gov/coloradotickfever/statistics.html.*)*

from 1987 to 2000, but that number has declined since for unknown reasons (possibly related in part to changes in testing and reporting practices).[21,27] CTFV disease cases typically occur from March through September, peaking in May or June. Males make up a higher percentage of reported cases than females. All age groups can be affected, but the highest incidence of reported CTFV disease appears to be in those of age >40 years.[21,24]

Clinical and Laboratory Features

CTFV has been isolated from patients with acute febrile syndromes, meningitis, and encephalitis in North America. The first published clinical descriptions of CTFV appeared as early as 1855, with identification of the virus in 1946 after isolation from human serum.[28,29]

Onset of symptoms typically occurs after an incubation period of 1 to 14 days, usually within 3 to 4 days. The most common CTFV disease symptoms include abrupt fever, chills, headache, myalgias, and/or fatigue. Sore throat, nausea, vomiting, abdominal pain, arthralgia, neck pain or stiffness, and/or rash (either maculopapular or petechial) can also occur. Approximately half of CTFV disease cases have a biphasic "saddleback" illness with a remission for 1 to 3 days followed by recurrence of fever and other symptoms. Neurologic complications such as meningitis and encephalitis can rarely occur, especially in children. Other rare complications of CTFV disease include disseminated intravascular coagulation, myocarditis, pericarditis, pneumonia, hepatitis, and epididymo-orchitis.[6,20,22,29–31]

In terms of laboratory findings, leukopenia (<4500 cells/mm^3) is common with a relative lymphocytosis and atypical lymphocytes. Moderate thrombocytopenia may also occur. Anemia is rare, though CTFV can infect both erythrocytic precursors and red blood cells, potentially for weeks. For those with meningitis or encephalitis, cerebrospinal fluid analysis can show a lymphocytic pleocytosis with mild elevation of protein and near-normal glucose.[6,20,22,29–33]

Most people with CTFV disease recover after 1 week, but prolonged fatigue (weeks to months) may occur in those over age 30 years. Up to 30% of CTFV disease cases may be hospitalized at some point in their illness. Death is rare, often being associated with disseminated intravascular coagulation or meningoencephalitis in children.[6,20,22,29–31]

Diagnosis

Suspicion for CTFV disease is warranted for any person with a CTFV disease–like illness with recent travel to and exposure in CTFV-endemic areas. CTFV infection can be diagnosed either molecularly by detection of CTFV nucleic acid (through reverse-transcriptase polymerase chain reaction [RT-PCR]) or serologically by detection of CTFV-specific immunoglobulin M (usually through indirect fluorescent antibody tests) and confirmatory CTFV neutralizing antibodies (usually through plaque-reduction neutralization testing). Given CTFV's ability to infect red blood cells for weeks, RT-PCR can often detect CTFV nucleic acid in the serum reliably for the first 2 weeks after symptom onset. On the other hand, antibody production is often delayed in CTFV infection, and CTFV antibody tests may not be positive until 2 to 3 weeks after onset of symptoms.[6,20,34,35] CTFV testing is available from some commercial laboratories and some state health departments, and at the US Centers for Disease Control and Prevention. State and local health departments can often assist with testing.

The differential diagnosis of CTFV-like illness includes other vector-borne diseases such as Rocky Mountain spotted fever, tick-borne relapsing fever, tularemia, and other arboviral diseases.

Treatment and Prevention

No specific antiviral therapy is available for CTFV disease, and treatment is supportive. Aspirin should be avoided because it may exacerbate the potential for hemorrhage associated with thrombocytopenia. Patients with severe symptoms may need to be hospitalized for rehydration and symptom management.[6,20,29] Ribavirin inhibited the growth of CTFV in vitro and ribavirin triacetate increased the survival of mice infected with CTFV, but no studies have been completed to determine these agents' efficacy or safety in humans.[36]

Tick-bite prevention strategies are most important in limiting CTFV disease in humans. People outdoors in endemic areas should use insect repellent on exposed skin, wear long-sleeved shirts and pants when feasible, avoid walking through high-brush areas, and perform tick checks once inside.[6,20]

Given the persistence of CTFV in bone marrow and red blood cells, persons infected with CTFV should not donate blood or bone marrow for at least 6 months after their illness.[6,20]

Salmon River Virus

It is unclear whether Salmon River virus is a distinct coltivirus or just a serotype of CTFV. It was isolated from a viremic patient with a CTFV-like illness and named for the area where infection was likely acquired: Idaho's Salmon River. Little is known about its epidemiologic, ecologic, or clinical features.[5]

Eyach Virus

Eyach virus is an antigenically related but distinct coltivirus from CTFV that was first isolated from *Ixodes* sp. ticks in France and Germany. Its ecologic cycle is not fully known, though its amplifying host is thought to be the European rabbit, and serosurveys have found Eyach virus–specific antibodies in rodents, sheep, goats, and deer in parts of Europe.[5–11] Eyach virus has been suspected of causing human neurologic disease (specifically polyradiculoneuritis and meningoencephalitis) because Eyach virus–specific antibodies were recovered in the serum of affected patients.[8] However, the actual virus was never isolated from these patients so causation is difficult to prove. Eyach virus–specific RT-PCR and antibody tests have been developed.

SEADORNAVIRUSES

Banna Virus

Banna virus is a presumably mosquito-borne seadornavirus endemic to Southeast Asia and China, and is the only known seadornavirus implicated in human disease to date.[3,5,12–16] It has been isolated from multiple mosquito species (e.g., *Aedes, Anopheles, Culex* spp.) throughout the region. Not much is known about its ecologic cycle or epidemiology. Banna virus was first isolated from the sera of febrile patients and the cerebrospinal fluid of encephalitic patients in the Xishuangbanna Prefecture of China's Yunnan Province in 1987.[12] Banna virus was later isolated from the sera of several febrile patients in China's Xinjiang Province, and Banna virus–specific antibodies were detected in several patients suspected of having Japanese encephalitis virus disease throughout eastern China.[13] Clinically, symptoms of Banna virus disease appear to include fever, myalgia, arthralgia, and/or encephalitis. However, the

full range of clinical disease and subsequent outcomes is not known. Banna virus–specific RT-PCR and antibody tests have been developed. There is no specific known treatment for Banna virus disease other than supportive care. Mosquito-bite prevention strategies such as applying insect repellent when outdoors, wearing long-sleeved shirts and pants when feasible, and using window screens or air conditioning when indoors may help prevent infection in endemic areas.

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150 Rotaviruses

Philip R. Dormitzer[a]

SHORT VIEW SUMMARY

Definition
- Rotavirus is a nonenveloped, double-stranded (ds)RNA virus that is the most important cause of dehydrating infant and childhood gastroenteritis.

Viral Structure and Replication
- The viral particle is a triple-layered icosahedron.
- Genome consists of 11 segments of dsRNA.
- The outer-layer glycoprotein VP7 and the spike protein VP4 mediate entry into cells and are the neutralization determinants.
- The virus binds cell surface carbohydrates.
- Cells are entered by viral penetration of cellular membranes.
- After the outer layer is removed, a subviral particle transcribes and extrudes messenger RNA.
- New subviral particles assemble in viroplasms in the cytoplasm, and virions are completed by addition of the outer-layer proteins in the endoplasmic reticulum.

Clinical Manifestations
- Gastroenteritis due to rotavirus is not readily distinguished from gastroenteritis caused by other agents on clinical grounds alone.
- Main symptoms are diarrhea and vomiting. Severe dehydration and death can result.

Pathogenesis
- The virus primarily infects epithelial cells at the tips of the intestinal villi.
- Multifactorial pathogenesis of rotavirus diarrhea includes malabsorption due to loss of intestinal epithelial cells, an enterotoxin, and enteric nervous system signaling to create a secretory state.

Serologic Classification
- Most rotaviruses that cause human disease are in group A.
- There is extensive diversity of antigenic types.
- Within group A, rotavirus has a dual serologic classification system, with G type based on VP7 and P type based on VP4.
- Genetic classification is starting to replace serologic classification.

Epidemiology
- Infection is universal in the first years of life.
- Infection may be symptomatic or asymptomatic.
- Severe illness is most common between 6 months and 2 years of age.
- Reinfection occurs throughout life.
- There is a large burden of illness in all socioeconomic settings, but mortality is concentrated in developing countries.
- As of 2016 rotaviruses caused approximately 128,500 deaths of children younger than 5 years each year, a reduction from approximately 538,000 in 2000 due, in part, to implementation of vaccines.[1]
- Exchange of strains and genome segments between human rotaviruses and animal rotaviruses maintains the diversity of circulating strains.

Immunity
- Partial protection from reinfection is provided by previous infection, with generally decreasing severity of subsequent infections.
- Serotype influences but does not determine protection from reinfection.
- VP4 and VP7 each contain serotype-specific and heterotypic neutralizing epitopes.
- Neutralizing antibodies block the functions of the outer capsid proteins in cell entry.
- Nonneutralizing immunoglobulin A against the middle-layer protein, VP6, can interfere with intracellular virus replication, as the antibody is transcytosed across intestinal epithelial cells.
- Humoral immunity appears to be the most important determinant of protection from infection.
- Innate and cellular immunity contribute to clearance of infection.
- Rotavirus interferes with the innate immune response by several mechanisms.

Diagnosis
- Specific virologic diagnosis is not necessary for routine clinical care but is useful for epidemiologic study, in complicated or prolonged cases, and to reduce unnecessary antibiotic use.
- Antigen can be detected readily in the stool, most commonly by enzyme-linked immunosorbent assay.
- There is increasing use of nucleic acid–based testing for epidemiologic investigations.

Therapy
- Therapy is primarily supportive and aimed at maintaining hydration until the infection resolves.
- No specific antiviral therapy is available.
- Mild and moderate dehydration can be treated effectively with oral rehydration solutions.
- Treatment of patients who have severe dehydration requires the use of intravenous fluids.
- In the context of malnutrition, zinc supplementation may be a useful adjunct to oral rehydration therapy.

Immunization
- Two live-attenuated, oral rotavirus vaccines have been licensed in multiple countries, another two have been licensed in India and achieved World Health Organization prequalification, and two more have national licenses in China or Vietnam.
- Where rotavirus vaccines have been introduced, they have dramatically decreased severe rotavirus gastroenteritis and substantially decreased severe dehydrating pediatric gastroenteritis overall.
- A rare complication of immunization, intestinal intussusception, led to the withdrawal of a previous rotavirus vaccine, but there is consensus that the benefits of immunization with the current rotavirus vaccines far outweigh the risk of intussusception.
- Progress is being made in distributing rotavirus vaccines in developing countries where mortality is concentrated, in part due to local production of new rotavirus vaccines.
- Despite lower efficacy in impoverished settings than in affluent settings, rotavirus vaccines have the potential for a large impact on infant mortality worldwide.

HISTORY AND OVERVIEW

Classification and Impact

Rotavirus is a genus within the Reoviridae, a family of nonenveloped, icosahedral animal viruses with double-stranded (ds), segmented RNA genomes. The family Reoviridae also includes reoviruses, which have occasionally been isolated from patients with respiratory illnesses but have not been established as a cause of human disease, and coltiviruses and seadornaviruses, which cause disease in humans (see Chapters 148 and 149). Rotaviruses are the most important cause of severe dehydrating gastroenteritis in children younger than 5 years in all socioeconomic groups and in all regions of the world. In 2016 they caused approximately

[a]Philip Dormitzer notes a potential conflict of interest because he is a Pfizer employee and shareholder.

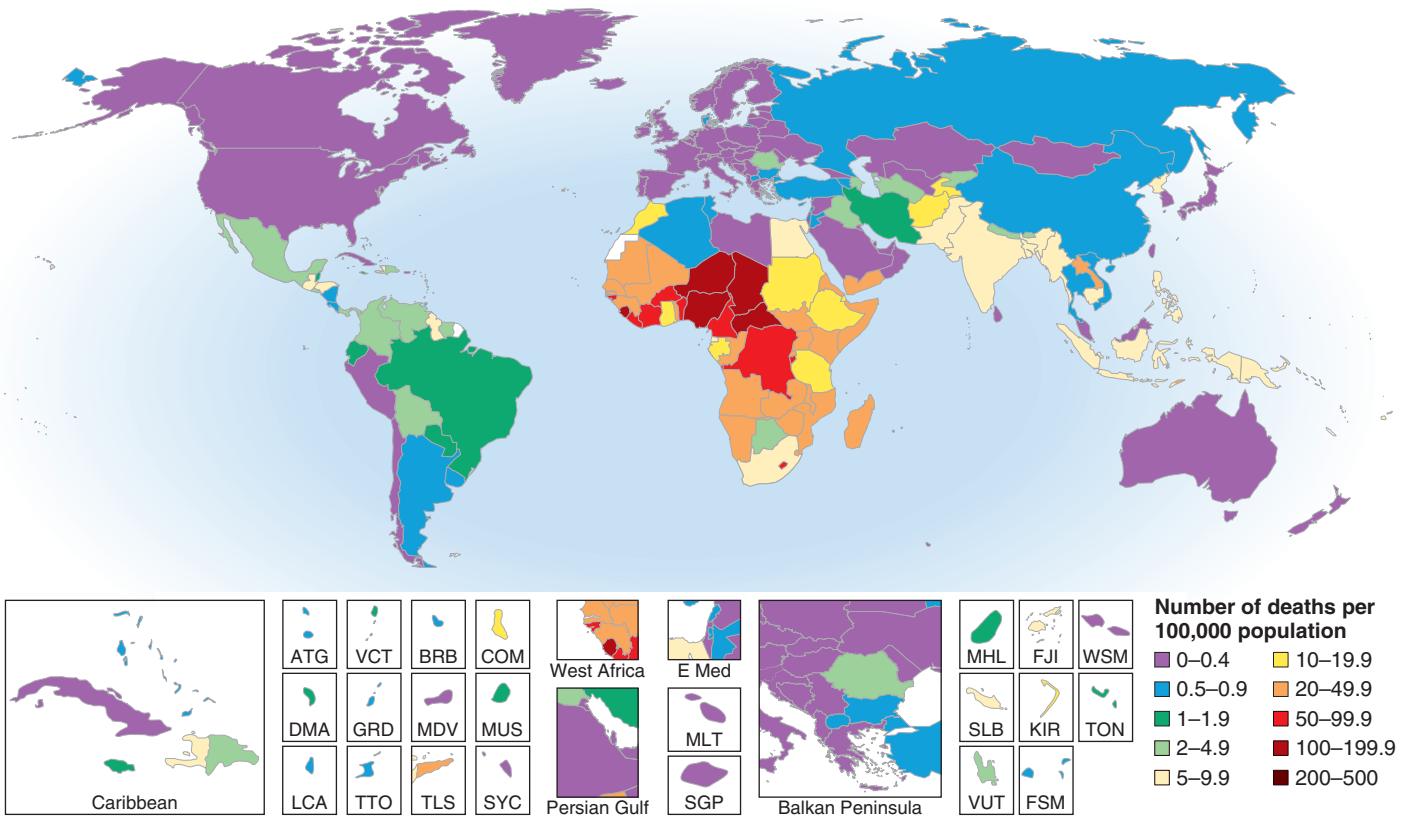

FIG. 150.1 Geographic distribution of rotavirus-associated mortality rates among children younger than 5 years in 2016. *(From Troeger C, Khalil IA, Rao PC, et al. Rotavirus vaccination and the global burden of rotavirus diarrhea among children younger than 5 years.* JAMA Pediatr. *2018;172:958–965.)*

238 million episodes of diarrhea and approximately 128,500 deaths of infants and children younger than 5 years, with approximately 104,733 of those deaths occurring in sub-Saharan Africa (Fig. 150.1).[1] Higher latitude and higher income correlate with a seasonal pattern of disease in cold and dry months; lower latitude and income correlate with less distinct seasonality, although neither geography nor income fully account for observed annual patterns.[2]

Discovery

Before the association of rotaviruses with human disease, etiologic agents were not identified in most cases of childhood gastroenteritis. In 1973 electron microscopic examination of duodenal biopsy specimens from six of nine children with acute gastroenteritis revealed similar viral particles, which were approximately 70 nm in diameter.[3] Morphologically, these particles were indistinguishable from viruses previously identified in specimens from mice and cows with diarrhea[4,5] and were designated "rotaviruses" because of their appearance in electron micrographs as wheels with spokes (Fig. 150.2). The antigenic similarity between the human and bovine agents was confirmed when an exchange of matched liquid stool samples and convalescent sera between a veterinary and a medical laboratory showed that antibodies in the sera of children and calves agglutinated the rotavirus particles in the stools of both species.[6] The human sera also neutralized the infectivity of the bovine agent, which had been adapted to growth in cell culture.[6]

Path From Basic Science to Vaccine Introduction

Since the discovery of the agent, understanding of rotavirus replication, pathogenesis, and immunity has relied on the study of strains that grow well in cell culture and of large and small animal models for rotavirus infection and disease. The ability to "mate" rotavirus strains by coinfecting cells to achieve reassortment of the 11 genome segments has allowed classic genetic studies. Extensive global epidemiology has relied on elaborate serology, now supplemented by nucleic acid–based diagnostics. This scientific background enabled the introduction of live-attenuated vaccines for human use. These vaccines greatly reduced the health impact of rotaviruses in the upper- and middle-income countries where they were first introduced, with evidence of both direct vaccine-elicited immunity and a herd effect.[7] The development of locally produced, less costly live-attenuated rotavirus vaccines promises more widespread rotavirus immunization for the most severely affected populations in developing countries.[8] The application of molecular and structural biology provides the scientific basis for the next generation of vaccines, which may overcome the lower effectiveness of the live-attenuated approach in low-income settings.[9]

VIRAL STRUCTURE AND REPLICATION

Structural Overview

The rotavirus virion is a nonenveloped icosahedral particle that is approximately 770 Å in diameter, excluding the VP4 spikes (Fig. 150.3A).[10] It consists of three concentric protein layers that encapsidate 11 segments of tightly packed dsRNA, together with polymerase and capping enzyme complexes. Each genome segment contains one or, in the case of genome segment 11, two open reading frames. These segments encode six structural proteins (VP1–VP4, VP6, and VP7) and six nonstructural proteins (NSP1–NSP6).

The innermost protein shell consists of 120 copies of VP2, arranged in an icosahedral lattice (T = 1 with two VP2 molecules making up each icosahedral asymmetrical unit).[10] The VP1 polymerase and VP3 capping enzyme are anchored inside the VP2 shell adjacent to pores at the icosahedral fivefold vertices. The middle layer consists of 780 copies of VP6, which form thick trimeric pillars in an icosahedral lattice (T = 13 *levo*). Although not exposed on the virus surface, VP6 is the target of the most abundant antibodies elicited by rotavirus infection. The genome, VP1, VP3, and the inner two protein layers make up the transcriptionally active, double-layered subviral particle (DLP) (see Fig. 150.3C *right*).

The thin outermost layer consists of 780 copies of a coat glycoprotein, VP7, and 180 copies of a spike protein VP4, which forms asymmetrical trimers that protrude from the virion (see Fig. 150.3A, D *left*).[10] One hundred and thirty-two aqueous holes perforate the outer and middle layers. VP4 and VP7 translocate the DLP across a host membrane and into the cytoplasm (see Fig. 150.3C). Both outer-layer proteins are neutralization antigens.[11] VP4 is the major cell attachment protein, a membrane penetration protein, and a determinant of virulence.[11–14] VP7 forms the icosahedral lattice of the outermost layer, which is in register with the VP6 lattice of the middle layer.

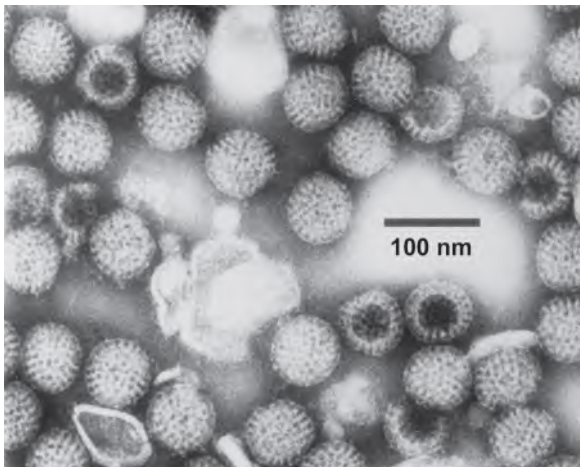

FIG. 150.2 Electron micrograph of negatively stained rotavirus virions in a stool filtrate from a child with gastroenteritis. The outer capsid appears as a thin rim surrounding stubby protrusions made up of VP6 trimers. "Empty" particles with dark centers lack genomic RNA. *(From Kapikian AZ, Kim HW, Wyatt RG, et al. Reoviruslike agent in stools: association with infantile diarrhea and development of serologic tests.* Science. *1974;185:1049–1053.)*

Inactivation

The rotavirus particle is physically hardy and resists inactivation by treatment with fluorocarbons, ether, and concentrations of chlorine typically used to treat sewage effluent and drinking water.[15] However, the particle is inactivated by calcium chelators and by antiseptic agents that contain relatively high concentrations of alcohols (>40%), free chlorine (>20,000 ppm), or iodophors (>10,000 ppm iodine).[16] A commonly used hand-sanitizing agent (Purell), which contains 62% ethanol, substantially reduces viable rotavirus carriage on fingertips,[17] and a disinfectant spray (Lysol), which contains 79% ethanol and 0.1% *o*-phenylphenol, prevents the experimental transmission of rotavirus from fomites to human volunteers.[18] Rotavirus survival in the environment is significantly decreased at high relative humidity.[15]

Priming for Infectivity

Efficient rotavirus infectivity requires priming of the virus by intestinal trypsin, which cleaves the spike protein, VP4, into an amino-terminal fragment, VP8*, and a carboxy-terminal fragment, VP5* (see Fig. 150.3B, C *left*, D *left*). The VP8* fragment contains a carbohydrate receptor-binding domain (lectin domain), and the VP5* fragment contains a β-barrel domain that interacts with membranes.[10] Cleavage into VP5* and VP8* triggers an initial rearrangement of VP4 trimers into an asymmetrical conformation (see Fig. 150.3D *left*) in which the three subunits form a "foot," buried under the VP7 shell with approximate threefold symmetry, and the β-barrel domain of VP5* of one subunit

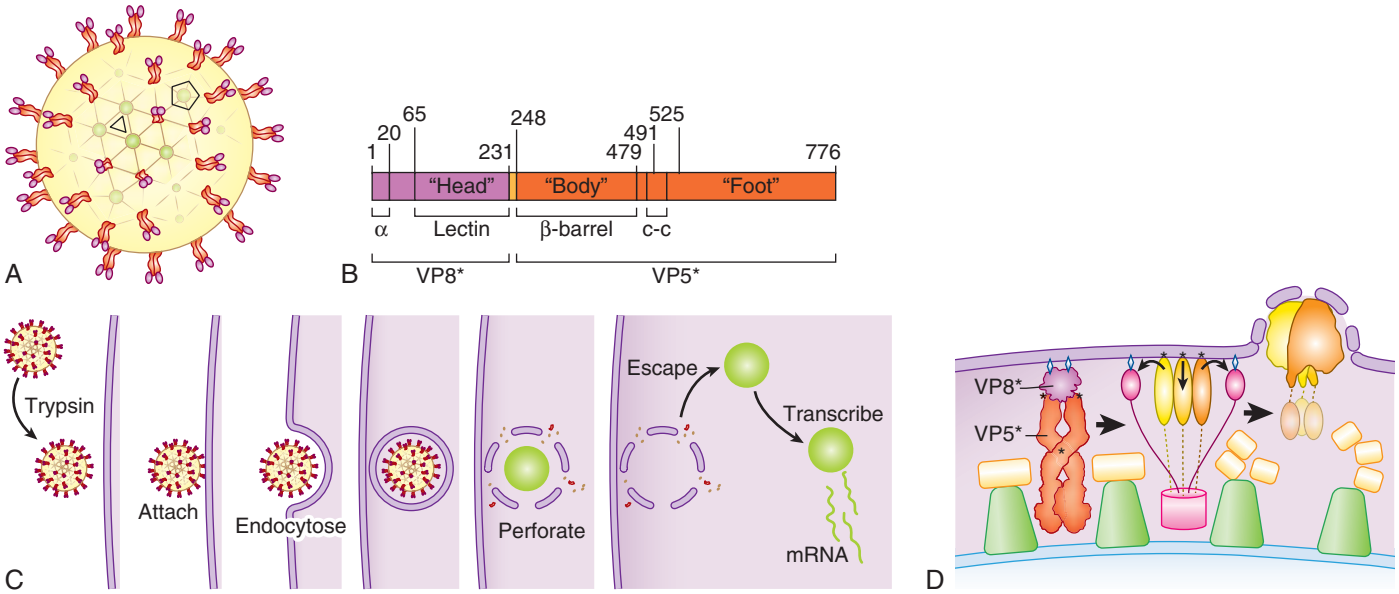

FIG. 150.3 The rotavirus particle and conformational transformations during entry. (A) Surface representation of the mature rotavirus virion. VP4 is in *magenta* (VP8*) and *red* (VP5*); VP7, in *yellow;* VP6 in *green*; *black triangle*—threefold symmetry axis; *black pentamer*—fivefold symmetry axes. (B) Domains of VP4. Numbers above the bar, amino-acid residues; lines immediately beneath the bar, substructures of cleaved VP4 (α: N-terminal helix, which anchors VP8* into the foot; *lectin*: receptor-binding domain; β-*barrel*: projecting domain of VP5*, which contains the membrane interaction loops; *c-c*: coiled-coil structure, which forms when VP5* folds back); *bottom lines:* trypsin cleavage fragments. (C) Entry pathway. The loss of VP4 and VP7 after endocytosis into a tightly fitting vesicle is concurrent with membrane perforation and converts the triple-layered virion into a double-layered, transcriptionally active particle with a VP6 *(green)* surface. (D) Proposed correlation of conformational changes in the mature VP4 spike with remodeling and breaking of a bilayer membrane. *Left:* mature spike, anchored into the VP6 layer *(green trapezoid)* by VP7 *(yellow rounded rectangle)*. VP4 is activated by cleavage to VP8* *(magenta)* and VP5* *(red*). *Black stars:* hydrophobic membrane interaction loops of the VP5* β-barrel domain; *blue diamonds:* glycolipid head groups to which the VP8* lectin domains attach; *blue curved bar:* VP2 layer; *purple curved bar*: cellular membrane. *Center:* Schematic diagram of the product of an initial conformational change, in which VP8* lectin domains dissociate from their positions covering the hydrophobic loops of VP5*, exposing them for interaction with the membrane. VP8* lectin domains are *magenta ovals;* VP5* β-barrel domains are *red, orange,* and *gold ovals* connected by flexible links to the foot *(pink cylinder). Right:* loss of Ca^{2+} causes VP7 *(yellow)* to detrimerize and dissociate from VP6, releasing VP5*, which folds back to its most stable, postcleavage conformation. The interaction between membrane and VP5* hydrophobic loops couples membrane distortion and perforation *(dashed lines)* to folding back of VP5*. VP5* coiled-coil and β-barrel domains are *red, orange,* and *gold irregular shapes;* VP5* foot domains are *light red, orange,* and *gold ovals. (Modified from Abdelhakim AH, Salgado EN, Fu X, et al. Structural correlates of rotavirus cell entry.* PLoS Pathog. *2014;10:e1004355. Courtesy Stephen C. Harrison.)*

forms a "stalk" that supports a "body," formed by the β-barrel domains of VP5* from each of the other two subunits.[10] The spike is topped by two "heads," formed by the VP8* lectin domains of two of the subunits.[10]

Receptor Binding and Human Susceptibility

The rotavirus cell entry pathway is complex, with entry-related conformational changes in VP4 and VP7, strain-dependent variation in use of cell surface receptors, and possibly strain-dependent variation in the use of different endocytic pathways.[19,20] The first rotavirus interaction with a cell generally occurs when the VP8* lectin domains bind a cell surface glycan (see Fig. 150.3B, C). Many rotavirus strains that cause human disease bind to the human histo-blood group antigens (HBGAs), which are present not only on the surface of erythrocytes but also on enterocytes and other cells.[21,22] The distribution of HBGAs in human populations is determined by polymorphism in glycosyltransferases, such as fucosyltransferase 2 (FUT2; for secretor status) and FUT3 (for Lewis type). The specificity of rotaviruses for different HBGAs varies with their P genotypes (determined by VP4).[22] Thus human populations vary in their susceptibility to infection by certain rotavirus strains. For example, those who are secretor negative appear to be resistant to disease caused by P8 rotaviruses and at lower overall risk of severe rotavirus gastroenteritis in the United States, where P8 rotaviruses are prevalent.[23–25] In Bangladesh nonsecretor status was found to protected against P4 rotavirus disease.[26] In contrast, many rotaviruses that infect nonhuman animals initially bind neuraminidase-sensitive terminal sialoside on cell membrane glycoproteins or glycolipids.[27] A variety of potential secondary receptors, such as heat shock protein 70 or integrins α2β1, αVβ3, or αXβ2, have been proposed for rotavirus, although the position of the putative binding sites for these ligands on VP4 and VP7 appear inconsistent with interactions before virus uncoating.[28]

Internalization Into Target Cells

Several rotavirus entry pathways have been proposed, such as direct plasma membrane penetration or uptake through dynamin-dependent, clathrin-dependent, or clathrin- and caveolin-independent endocytosis.[20,29,30] Rotavirus entry studies are complicated by many nonproductive internalizations accompanying every productive entry event and by potential differences in entry pathway based on virus strain or cell type. The entry of single, fluorescently labeled viruses has been studied in cultured monkey kidney epithelial cells, with the rapid cytoplasmic diffusion of rotavirus DLPs after separation from the viral outer layer used as a marker for productive entry. In these experiments, the VP4 spikes appear to mediate the invagination of virus adherent at the plasma membrane into tight-fitting vesicles (see Fig. 150.3C) that are not consistently associated with the endosomal marker Rab5, the clathrin-associated adaptor protein AP-2, or dynamin.[31]

Conformational Changes Linked to Membrane Perforation and Escape

Endosomal acidification is not required for rotavirus entry, as it is for many other viruses. Instead, loss of calcium from the tight-fitting vesicle formed around the virus as it is invaginated appears to be a key trigger for rotavirus molecular rearrangements that lead to cell entry (see Fig. 150.3C, D).[32] In low calcium environments, calcium is lost from the VP7 intratrimer interface, and the VP7 trimers dissociate, releasing clamplike contacts with VP6 so that VP7 is shed from the virion.[33,34] When VP7 uncoating occurs in the presence of membranes, cleaved VP4 rearranges to an inferred intermediate conformation that binds the membranes (see Fig. 150.3D *middle*).[35] This intermediate conformation resolves into a post–membrane-penetration state in which the VP5* fragments form an umbrella-shaped, threefold symmetrical, folded-back trimer (see Fig. 150.3D *right*).[36] During these conformational changes, the hydrophobic apex of VP5* is translocated from one end of the molecule to the other.[36] This motion resembles the fold-back rearrangements of enveloped virus fusion proteins, which mediate fusion between a virus envelope and a cellular membrane. Loss of membrane perforation by recoated rotaviruses, as measured by α-sarcin entry into cells, when the VP5* apices are mutated to reduce their hydrophobicity, suggests that the "jackknifing" of VP5* breaches the cellular membrane.[14] Escape of DLPs from the small vesicles that form around invaginating rotavirus virions also requires intact hydrophobic VP5* apices, suggesting that the VP5* rearrangement disrupts this small vesicle to release DLPs into the cytoplasm (see Fig. 150.3C).[14,31] The failure of particles with cross-linked VP7 to perforate membranes or escape from the small vesicles suggests that release of the VP7 "clamp" is required for the VP5* rearrangement to occur (see Fig. 150.3D).[31,37]

Transcription From Double-Layered Particles and Preferential Translation of Viral mRNA

The loss of VP7 leads to conformational shifts deeper in the particle, near the polymerase complex below the channels at the fivefold vertices, to activate the transcriptase activity of the rotavirus DLP so that, once in the cytoplasm, the DLP extrudes capped, nonpolyadenylated messenger RNA (mRNA) through channels at its fivefold icosahedral vertices (see Fig. 150.3C *right*).[34,38,39] The genome remains encapsidated throughout transcription. The translation of viral mRNAs is enhanced by circularization, mediated by the specific binding of the rotavirus nonstructural protein NSP3 to a four-nucleotide consensus sequence at the 3′ end of viral mRNA and to the cellular cap-binding protein eIF4G.[40–42] Thus NSP3 substitutes for a cellular polyadenylic acid–binding protein to favor the translation of the nonpolyadenylated viral mRNAs over polyadenylated host mRNAs.

Genome Packaging and Assortment

Rotavirus genome replication must overcome two major challenges: One copy of each of 11 different RNAs must be selected for packaging, and the newly synthesized dsRNA must be sequestered so that it does not induce an interferon (IFN) response. The mechanism of selective packaging remains obscure but may involve base-pairing interactions between the nascent genome segments; the mechanism of genome sequestration is better understood.[43] The viral polymerase, VP1, specifically binds rotavirus mRNAs by recognizing a consensus sequence (UGUG) near their 3′ ends and holds the mRNAs in inactive complexes.[44] The mRNA shifts to a position that permits negative-strand synthesis only when the polymerase binds to the inner face of a decamer of the inner-shell protein, VP2.[45] The dependence of negative-strand synthesis on VP1-mRNA complexes binding to the inner-shell protein ensures that double-stranded genomic RNA is synthesized within a nascent particle.

Viroplasm Formation

DLPs are assembled in cytoplasmic viral "factories," termed *viroplasms.*[43] Viroplasms are associated with intracellular lipid droplets and form through the interaction of rotavirus nonstructural proteins NSP2 and NSP5 and through lattice formation by NSP2 when both NSP2 and NSP5 are phosphorylated by a cellular kinase, casein kinase 1α, and by autokinase activity.[46,47] NSP2 is an octomeric doughnut-shaped structure with RNA-binding, helix-destabilizing, autokinase, nucleoside triphosphatase, and nucleoside diphosphate kinase–like activities.[47] Activities proposed for NSP2 include mediating specific interactions between rotavirus RNAs to drive genome segment assortment, acting as a molecular motor to drive replicating mRNA into the nascent particles, and maintaining nucleotide pools in viroplasms during replication.[48–50] NSP5 is a metalloprotein that binds an iron-sulfate cluster and also a phosphoprotein that has autokinase activity.[51,52] NSP5 probably has a regulatory role, competing with single-stranded RNA to bind in grooves of the NSP2 octamer, competing with VP6 for binding to VP2, and binding RNA.[53,54] Once VP6 is added to the nascent particle, the newly formed DLP can transcribe mRNA, amplifying virus yield. Newly transcribed rotavirus mRNA enters two pools: mRNA that remains sequestered within viroplasms is replicated and packaged as genome segments; mRNA that escapes viroplasms is translated.[55]

Reverse Genetics Systems

Despite the barrier imposed by the need to introduce transfected mRNA into viroplasms for replication into packaged rotavirus double-stranded genome segments, recent advances in rotavirus reverse genetics systems now allow the relatively efficient incorporation of recombinant genes

into infectious rotavirus genomes.[56] Previous rotavirus reverse genetics systems required the use of helper viruses or genes encoding accessory proteins to achieve reliable rotavirus rescue from DNA.[57] However, increasing the amounts of plasmids encoding NSP2 and NSP5 relative to the amounts of plasmids encoding the other genome segments in the transfection mixtures has allowed recovery of infectious rotaviruses expressing fluorescent markers after the transfection of plasmids encoding the rotavirus genome segments.[56] These advances in rotavirus reverse genetics promise accelerated basic discovery and, potentially, rationally attenuated vaccine strains.

Outer Capsid Assembly

After genome replication and DLP assembly, the outer capsid is added through an unusual maturation process. The DLP binds the cytoplasmic tail of NSP4, a rotavirus NSP that is resident in the membranes of the endoplasmic reticulum (ER) and viroplasm-associated vesicles, and then buds into the ER lumen, transiently acquiring an envelope.[58] VP7, also resident in the ER, mediates removal of the transient envelope and binds the DLP, locking the VP4 spike protein in place.[59,60] Thus rotavirus penetrates membranes twice. During entry rotavirus penetrates a cellular membrane through a mechanism involving rearrangements of cleaved VP4 as the outer capsid disassembles (see Fig. 150.3C, D); during maturation rotavirus penetrates an envelope derived from the ER through a mechanism that involves VP7 and possibly NSP4, as the outer capsid assembles. Rotavirus egress from infected enterocytes is accomplished by release from the apical surface after vesicular transport from the ER by a pathway that bypasses the Golgi apparatus.[61]

CLINICAL MANIFESTATIONS

Typical Clinical Illness

Infants and young children with diarrhea caused by rotavirus are more likely to have severe symptoms and become dehydrated than patients with diarrhea related to other common enteric pathogens.[62,63] The clinical manifestations of rotavirus gastroenteritis in humans have been studied in experimental infections in adults. In one such study 4 of 18 adult volunteers developed vomiting 1 to 3 days after oral administration of a virulent rotavirus strain.[64] This was followed by diarrhea lasting 1 to 4 days and associated with anorexia, crampy abdominal pain, and low-grade fever. Viral shedding in stool was detected for 6 to 10 or more days. Two-thirds of the adult volunteers developed serologic evidence of infection without disease. Similarly, most naturally occurring rotavirus infections of adults are asymptomatic, manifested only by a rise in antibody titer.[65] However, rotavirus infection of adults can also cause severe and even fatal disease.[66]

Observational studies of children in North America hospitalized with rotavirus gastroenteritis reveal a similar, although more severe, pattern of disease.[62,63,67] Rotavirus gastroenteritis in children generally begins with vomiting and fever, which lasts 2 to 3 days, and progresses to profuse watery diarrhea, which continues for 4 to 5 days. Vomiting is more common and prolonged with rotavirus gastroenteritis than with pediatric gastroenteritis caused by most other agents.[67]

Clinical Laboratory Findings

Laboratory findings in children hospitalized with rotavirus gastroenteritis reflect isotonic dehydration and include high urine specific gravity and metabolic acidosis.[62,67] Rotavirus gastroenteritis is not generally associated with leukocytosis but is sometimes accompanied by a mild elevation in aminotransferase and uric acid levels.[62,67] Liquid stools from children with rotavirus diarrhea usually do not contain blood or fecal leukocytes,[62,67] although the presence of fecal leukocytes does not exclude rotavirus as a cause.[63] Typically, virus is detected in stools of children by antigenic assays for 4 to 10 days after the onset of symptoms.[62] When a sensitive reverse-transcriptase polymerase chain reaction (RT-PCR) assay is used, shedding of viral RNA can be detected for up to 57 days from immunocompetent children, although this does not necessarily indicate shedding of infectious particles.[68]

Spectrum of Disease Severity

Dehydration and severe electrolyte abnormalities leading to cardiovascular failure are the most common proximate causes of death from rotavirus gastroenteritis.[69] Seizures and aspiration of vomitus may also lead to death.[69] The spectrum of illness after rotavirus infection of infants and children also includes mild gastroenteritis and asymptomatic infection. In some neonatal nurseries, difficult-to-eradicate endemic rotavirus strains asymptomatically infect neonates year round.[70,71] A common VP4 type (P[6]) and genetic stability over time suggest that these nursery strains may be less virulent than most circulating strains,[70] although maternally transmitted immunity or maturational resistance may also protect the neonates from disease. Because outbreaks of rotavirus diarrhea in neonatal nurseries also occur, any component of maturational resistance cannot be absolute.[72]

Extraintestinal Manifestations and Disease in Immunodeficiency

Rotavirus also has extraintestinal manifestations.[73] Viremia, which is common in symptomatic and asymptomatic rotavirus infection, has unknown clinical significance in immunocompetent children.[74] In immunocompromised children rotavirus infection has been associated with chronic diarrhea and extraintestinal infection. Conditions associated with chronic rotavirus infection include X-linked agammaglobulinemia, cartilage hair hypoplasia, and DiGeorge syndrome.[75–78] Rotavirus shed from chronically infected children often has altered genome segments.[76,78] Immunohistochemical staining for rotavirus structural and nonstructural proteins in autopsy specimens has demonstrated rotavirus replication in the hepatocytes and renal tubular cells of immunodeficient children with chronic rotavirus infection at the time of death.[75] Relatively prolonged rotavirus shedding and severe gastroenteritis have been noted in both children and adult inpatients with immunosuppression related to bone marrow or renal transplantation.[79–82] Rotavirus is not more common or severe in children with human immunodeficiency virus (HIV) disease, and rotavirus immunization is not contraindicated for infants infected with or exposed to HIV.[83,84]

Other Disease Associations

Rotavirus has been detected in association with a number of syndromes other than gastroenteritis, including respiratory tract infections,[85] necrotizing enterocolitis,[86] pneumatosis intestinalis,[87] myocarditis,[88] seizures,[89] and meningoencephalitis.[89] Because rotavirus infection is universal, these associations may be coincidental rather than causative, although an 18% to 21% decrease in the incidence of seizures requiring hospitalization or emergency room care during the year after rotavirus immunization suggests that the link between rotavirus infections and seizures could be causal.[90] Although intestinal intussusception has been temporally associated with immunization with rotavirus vaccines (see "Immunization"), intestinal intussusception does not appear to be associated with natural rotavirus infection.[91]

Proposed Links to Autoimmunity

Links between rotavirus infection and the subsequent development of autoimmune disease have been proposed. Although some strains of rotavirus can cause biliary atresia in mouse models, and group C rotavirus has been detected in liver tissue from biliary atresia patients, rotavirus has not been convincingly demonstrated to cause this condition in humans.[92] Serologic evidence of more frequent rotavirus infection has been associated with the development of celiac disease in predisposed children.[93] A potential for molecular mimicry between rotavirus peptides and pancreatic islet autoantigens led to a proposal that rotavirus infection can be a trigger for type I diabetes mellitus.[94] To date the introduction of effective rotavirus immunization has not provided evidence of a causal link between symptomatic rotavirus infection and subsequent type 1 diabetes mellitus, celiac sprue, or primary biliary atresia.[95,96]

PATHOGENESIS

Pathologic Findings in Infection

The pathogenesis of rotavirus diarrhea is complex and incompletely understood, with potential roles for a viral enterotoxin, malabsorption related to mucosal damage and depression of disaccharidases, and secretion mediated by the enteric nervous system (ENS). Postmortem examination of the gastrointestinal (GI) tract of gnotobiotic pigs with diarrhea after experimental infection with a virulent human rotavirus

strain demonstrated that virus replicates primarily in the villous epithelium of the small intestine.[97] This pattern is consistent with the patchy villous epithelial distribution of rotavirus antigen noted after immunofluorescent staining of duodenal biopsy specimens from children with severe gastroenteritis.[98] Light microscopic examination of such duodenal biopsy specimens reveals shortened and blunted villi with a cuboidal epithelium, crypt hypertrophy, and mononuclear cell infiltration of the lamina propria.[3,99]

Human Intestinal Enteroid Infection Model

Human intestinal enteroids (HIEs) derived from human intestinal crypt domains can be differentiated into three-dimensional cultures that recapitulate elements of small intestinal architecture, contain multiple intestinal cell types, and support the efficient replication of human rotaviruses.[100] Human rotaviruses can replicate in HIEs from the duodenum, jejunum, and ileum; infect HIEs with villus-like architecture in preference to HIEs with cryptlike architecture; and infect both enterocytes and enteroendocrine cells.

Correlations Between Pathologic and Clinical Findings

The severity of diarrhea in children with rotavirus gastroenteritis correlates with the degree of mucosal damage, which suggests that malabsorption related to loss of absorptive cells may contribute to rotavirus diarrhea late in infection.[99] However, in experimentally infected gnotobiotic pigs, diarrhea precedes villous atrophy.[97] Similarly, small intestinal biopsy specimens from children with relatively mild rotavirus gastroenteritis do not consistently display histologic changes, probably reflecting patchy epithelial injury.[101] These observations indicate that potentially absorptive villous epithelial cells remain despite net fluid losses and that factors other than destruction of the intestinal epithelium are also important in the pathogenesis of rotavirus gastroenteritis.

Physiology of Rotavirus Diarrhea

In several animal models, rotavirus infection induces a net secretion of fluid, sodium, and chloride from intestinal segments.[102] Glucose cotransport of electrolytes is inhibited,[103] although the degree of inhibition in humans does not prevent rehydration with oral solutions. Decreased disaccharidase activity makes less glucose available for cotransport.[3,103] Changes in the molecular-weight distribution of absorbed polyethylene glycol in children with rotavirus gastroenteritis indicate an increase in paracellular permeability.[104] Experiments in cell culture suggest that disruption of tight junctions by VP8* or NSP4 may mediate this increased permeability.[105,106]

Viral Enterotoxin

The rotavirus nonstructural glycoprotein NSP4 is an enterotoxin that causes diarrhea in some animal models.[107] The fragment of NSP4 associated with this activity forms oligomers through a tetrameric or pentameric coiled-coil and raises cytoplasmic calcium levels, leading to a secretory state by activating a calcium-dependent chloride channel of the TMEM16 (transmembrane proteins of unknown function 16A) family.[108–111] Several mechanisms have been proposed for NSP4's effect on cytoplasmic calcium levels. Most NSP4 is resident in the ER membrane, and residues 47 to 90 have a viroporin-like activity that could disrupt the ER membrane, releasing calcium stores in infected cells.[112] In addition, an NSP4 peptide corresponding to residues 112 to 175 and intact NSP4 in lipoprotein complexes are released from rotavirus-infected cells and could affect uninfected cells.[113,114] The application of an NSP4 fragment (residues 95–146) to HIEs leads to swelling of the HIE lumens, an indicator of fluid movement from the basolateral to the apical surfaces of the HIE cells.[100] Extracellularly applied NSP4 fragments stimulate phospholipase C to produce inositol-1,4,5-triphosphate, which leads to calcium influx and release of calcium from intracellular stores.[115] Other mechanisms proposed for NSP4 enterotoxin activity include inhibition of sodium absorption by the epithelial sodium channel ENaC and by the glucose-coupled sodium cotransporter SGLTI and interference with lactase function.[111,116,117] Orally administered antibodies against NSP4 protect mice from rotavirus diarrhea, although actively acquired anti-NSP4 antibody fails to protect gnotobiotic piglets from challenge, and the role of an extracellular enterotoxin in human rotavirus gastroenteritis is not yet established.[107,118]

The Enteric Nervous System in Rotavirus Gastroenteritis

The ENS has a role in the pathogenesis of rotavirus gastroenteritis. Treatment of rotavirus-infected mice with lidocaine (a sodium channel–blocking anesthetic), ondansetron or granisetron (serotonin receptor antagonists), or a vasoactive intestinal peptide receptor antagonist attenuates diarrhea.[119–121] The ability of racecadotril, an enkephalinase inhibitor, to attenuate rotavirus diarrhea in children confirms that ENS-mediated secretion contributes to the pathogenesis of diarrhea in humans and is a target for therapeutic intervention (see "Therapy").[122] Secretion of nitric oxide, a mediator of intestinal motility, from NSP4-exposed human epithelial intestinal cells (HT-29) in culture, secretion of serotonin from an NSP4-exposed human midgut carcinoid enterochromaffin cell line (GOT1), and reduction of serotonin secretion from rotavirus-infected GOT1 cells in the presence of small interfering RNA that inhibits NSP4 expression suggest a potential role for the ENS in rotavirus-mediated intestinal hypermotility and vomiting and a link between the roles of NSP4 and the ENS in rotavirus pathogenesis.[121,123,124]

SEROLOGY AND CLASSIFICATION

Overview

The diversity of rotaviruses, their ability to exchange genome segments encoding antigenic determinants, and changing diagnostic technology have led to evolving serology and classification systems. Rotavirus strains have been classified serologically into serogroups, subgroups, G serotypes, and P serotypes, and genetically into electropherotypes, species, genogroups, G genotypes, and P genotypes. To organize the increasing volume of whole-genome rotavirus sequence data, in 2008 the Rotavirus Classification Working Group (RCWG) developed a nucleotide sequence–based complete genome classification system for group A rotaviruses.[125]

Rotavirus Groups

The broadest designation is the group, also referred to as serogroup or species. Historically, groups have been defined serologically, based on the cross-recognition of rotavirus particles by serum antibody obtained from parenterally hyperimmunized animals.[126] Serogroup determinants are predominantly located on VP2 and VP6 (the innermost and middle-layer proteins). More recently, a VP6 amino-acid sequence identity cutoff of 53% has been proposed as an alternative criterion for differentiating rotavirus groups or species.[127] Genetic exchange has not been observed between members of different groups. To date nine rotavirus groups (A–I) have been identified.[128] Groups A, B, and C cause disease in humans and other animals; groups D through I have been described only in nonhuman animals. Group A is the most important clinically, as group A viruses cause the endemic gastroenteritis of children; groups B and C have been associated with epidemics of gastroenteritis affecting all ages. Group B includes the strain ADRV (adult diarrhea rotavirus), which has been associated with large outbreaks of severe diarrhea in adults in China.[129] Group C viruses have been associated with less severe gastroenteritis in both children and adults.[130,131]

Binomial Serology of Group A Rotaviruses

Within group A, rotavirus serotypes have been defined primarily on the basis of reciprocal cross-neutralization of rotavirus strains by antibodies from immunized mice. Initially, rotavirus serotypes were designated by a unitary system, which primarily reflected neutralizing antibodies directed against VP7. This system did not fully explain cross-neutralization patterns because VP4 also contains neutralization determinants.[132,133] As a result, group A rotaviruses are now classified into serotypes with a binomial nomenclature, in which neutralization by antibodies against VP7 defines "G" serotype (for glycoprotein antigen), and neutralization by antibodies against VP4 defines "P" serotype (for protease-sensitive antigen). Further complicating the serology, VP7

and VP4 are each targets both of homotypic antibodies that neutralize only within serotype and of heterotypic antibodies that neutralize several serotypes.[133,134] Serotype-specific epitopes of VP4 are predominantly found on the variable VP8* lectin domain, at the tips of the spikes, and heterotypic epitopes are predominantly found on the more conserved β-barrel domain, in the body and stalk of the spikes (see Fig. 150.3D *left*).[10,12,36] Immunodominant serotype-specific and heterotypic neutralizing epitopes of VP7 straddle the calcium-containing interface between the subunits of each VP7 trimer, and additional neutralizing epitopes are found at an interdomain boundary within each VP7 subunit.[33]

Genetic Classification of Group A Strains

Early attempts at genetic classification grouped rotaviruses according to RNA genome electropherotype, which distinguished group A strains as "long," "short," and "supershort" on the basis of the electrophoretic mobility of genome segments 10 and 11. Rotavirus whole-genome analysis indicates that there are two main genogroups of human group A rotaviruses, each of which contains viruses with overall genome similarity.[135] The Wa-like human rotavirus genogroup appears to share origins with porcine rotaviruses, and the DS-1–like genogroup appears to share origins with bovine rotaviruses.[135] In the RCWG genetic classification, a separate genotype is assigned to each of the 11 genome segments. Gx, P[x], Ix, Rx, Cx, Mx, Ax, Nx, Tx, Ex, and Hx designate the genes that encode VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5/6, respectively.[125] The RCWG has proposed a strain-naming convention in which strains are named "RV serogroup/species of origin/country of identification/common name/year of identification/G- and P-type."[136]

Diversity of Group A Strains

Because the sequence of the genome segment encoding VP7 accurately predicts G serotype, genotyping provides a practical surrogate for G serotype determination. As of December 2018 the RCWG listed 36 accepted G types (G1–G36) (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg). At the time of vaccine introduction, five G types (G1–4 and G9) comprised 88% of human rotavirus isolates.[137] Since vaccine introduction, G12 viruses have emerged in the United States and other countries.[138] Substantial geographic and temporal variation exists in strain distribution. For example, G8 rotavirus strains are prevalent in high-rotavirus-mortality countries in Africa and therefore have more clinical significance than their global prevalence would suggest.[138]

To date at least 14 P serotypes (with serotypes 1, 2, and 5 each divided into subtypes with letter designations, such as P1A) have been identified.[125,137] Immunologic reagents for determining P serotype are limited, and P serotyping is complicated by cross-reactivity between P serotypes. Therefore P genotyping is more commonly used for classification. As of December 2018, 51 accepted P genotypes (P[1] to P[51]) were listed in the RCWG database. Unlike G serotypes, which have a one-to-one correspondence with G genotypes, some P serotypes include more than one P genotype.

In strain descriptions the genotype is enclosed in brackets after the serotype designation. Thus G2P1B[4] refers to a virus of G serotype 2, P serotype 1B, and P genotype 4. Two P genotypes, P[4] and P[8], cause most human rotavirus disease. Reassortment between human and animal rotavirus strains, antigenic drift, and the occasional introduction of animal rotaviruses into the pool of viruses circulating among humans provide a continuous introduction of genetic diversity, necessitating ongoing surveillance to determine whether rotavirus vaccines will require strain changes in the future for continued efficacy.[135]

EPIDEMIOLOGY

Universality of Infection

Rotavirus infection is universal, and almost all children acquire serum antibody against the virus in the first 2 or 3 years of life.[139] Severe gastroenteritis caused by rotavirus most commonly affects infants and children between 6 months and 2 years of age,[140] although in lower socioeconomic populations the peak of illness may be somewhat earlier.[140–142] The age at greatest susceptibility to severe disease may be bracketed by the waning of maternally transferred passive immunity and the maturation of the GI tract at about 6 months and by the acquisition of active immunity related to natural infection later in childhood.

Mortality and Global Impact

As of 2016 rotavirus remained the most common cause of severe dehydrating diarrhea in infants and children younger than 5 years in low-, middle-, and high-income countries, responsible for approximately 28.8% of deaths due to diarrhea in this age group.[1] The estimated 128,500 childhood deaths in 2016 is a substantial decrease from the estimated 453,000 childhood deaths in 2008.[1,143] A number of factors may be responsible for the decrease, including improvements in sanitation and nutrition, better access to treatment, more widespread use of oral rehydration solutions, reduced rates of coinfections, and the introduction of rotavirus vaccines. Although rotavirus gastroenteritis is common in both high- and low-income countries, mortality is concentrated in low-income countries, with approximately 104,733 of the rotavirus-associated deaths in 2016 occurring in sub-Saharan Africa (see Fig. 150.1).[1]

Health Care Utilization and Impact in High-Income Countries

The physical hardiness of the rotavirus particle, the high particle concentration in stool (up to 10^{11} particles/mL),[144] and the small minimum infectious dose (ID_{50} = 10 focus-forming units)[145] account for the weak association between the incidence of rotavirus gastroenteritis and the level of economic development or hygiene. Before the introduction of rotavirus vaccines in 2006, rotavirus was a major cause of morbidity and health care costs in developed countries. For example, in the prevaccine era in the United States, approximately 3 million cases of childhood rotavirus gastroenteritis were responsible for approximately 735,000 physician visits, 27,000 hospitalizations, 20 to 40 deaths, $319 million in direct health care costs, and $893 million in total economic costs to society each year.[146,147] Since rotavirus vaccines were introduced in 2006, there has been a large and sustained decrease in pediatric rotavirus gastroenteritis. By 2017 US pediatric hospitalizations due to rotavirus had declined by 80%, with an estimated annual savings in US direct health care costs attributable to rotavirus and acute gastroenteritis of approximately $121 to $231 million per year.[148]

Seasonality

Rotavirus gastroenteritis has been considered a winter disease, based on its historic seasonality in many temperate regions such as North America, where annual rotavirus epidemics typically began in the fall in the Southwest, spreading north and east, reaching the eastern seaboard by late winter, and tapering off in the spring.[149] Since the introduction of rotavirus immunization, this pattern has been disrupted with delayed or absent onset and shorter duration of the rotavirus season in the western, midwestern, and northern regions of the United States.[148] In lower-income regions and the tropics, year-round infection is a more common pattern.[2] During a rotavirus season, multiple strains, representing several P and G types, usually circulate. In a single region the dominant strains differ from year to year; in the same year the dominant strains differ regionally.[150]

Transmission

The best-documented and dominant mode of rotavirus transmission is fecal-oral.[145] However, the more limited effect of hygiene on the incidence of viral gastroenteritis than bacterial gastroenteritis and the universality of rotavirus infection early in life are more typical of pathogens with respiratory spread, suggesting that transmission may not be exclusively fecal-oral. Experiments on the transmission of rotavirus gastroenteritis (epizootic diarrhea of infant mice) between cages of mice support the possibility of airborne spread.[151] Although rotavirus can be foodborne, it is not a major cause of foodborne illness.[152] Transmission by contaminated water has also been documented.[129]

Risk Factors for Disease

Attendance at daycare centers is a risk factor for pediatric hospitalization due to rotavirus gastroenteritis.[153] The illness can spread in families and

may cause gastroenteritis in the adult caregivers for small children.[154] It is likely that the large viral inoculum ingested by these caregivers is, in part, responsible for illness despite some level of acquired immunity. Rotavirus outbreaks have occurred in nursing home populations and sometimes have resulted in fatalities.[66] Rotavirus is an important cause of nosocomial infection; in addition to the asymptomatic infections associated with rotavirus endemics in neonatal nurseries, rotavirus causes symptomatic hospital-acquired outbreaks associated with circulation of rotavirus in the community.[155] Rotavirus also can cause traveler's diarrhea.[156]

Veterinary and Zoonotic Disease

Rotavirus is a major veterinary pathogen, causing disease in infant cattle, sheep, swine, and camels, as well as in adult chickens and turkeys and in domestic pets, such as cats and dogs. In addition to the remote shared ancestry of major human and nonhuman animal rotavirus lineages, there is evidence of low-level ongoing zoonotic rotavirus disease.[135,157] In addition, some human rotaviruses have individual genome segments that are closely related to those of animal rotaviruses, suggesting reassortment between viruses with different primary hosts.[135] Nevertheless, in nonnative hosts most rotavirus strains are attenuated and do not spread efficiently.

IMMUNITY

Protection From Reinfection by Natural Infection

The immune response to rotavirus is complex and redundant: innate, cellular, and humoral mechanisms contribute to clearance of infection, and humoral immunity has a dominant role in the prevention of reinfection. The observation that natural rotavirus infection, whether symptomatic or asymptomatic, provides partial protection from subsequent episodes of rotavirus gastroenteritis guided the development of the current generation of vaccines. For example, asymptomatic infection of neonates with nursery strains of rotavirus protects against subsequent severe rotavirus gastroenteritis but not against asymptomatic reinfection or mild-to-moderate disease.[158] In a prospective observational study, 200 Mexican infants were monitored for rotavirus infection from birth to 2 years of age. Protection from subsequent moderate or severe rotavirus diarrhea was 87% after one natural infection of any severity and 100% after two natural infections.[139]

The duration of immunity to rotavirus is limited, with repeated symptomatic rotavirus infections occurring (generally with decreasing severity) in both children and adults.[139,159] In isolated human communities with limited prior exposure to rotaviruses, explosive epidemics of rotavirus diarrhea with high attack rates in adults are seen.[66,160] Although some experimental animals, including mice, show maturational resistance to rotavirus disease,[151,161] these clinical observations suggest that maturational resistance is less significant in humans and does not fully account for the lower incidence of severe disease in older children and adults. Rather, repeated asymptomatic or mildly symptomatic episodes of rotavirus gastroenteritis throughout life appear to be important for maintaining immunity.[65,162]

Protection by Antibody

In experimental studies of passively transferred immunity to rotavirus in mice, circulating neutralizing antibody does not protect against disease; however, the presence of sufficient levels of neutralizing monoclonal or colostrum-derived antibody in the gut does protect.[163,164] In immunized mice the appearance of immunoglobulin A (IgA) in the gut correlates with clearance of infection.[165] Passive infusion of neutralizing antibodies into monkeys protects from experimental challenge and, at high dose, results in detectable antibody in stool.[166] These experimental results corroborate the observation that, in naturally exposed children, preexisting rotavirus-specific serum IgA and fecal IgA, which reflect duodenal IgA, are associated with protection from disease.[162,167,168] Postimmunization rotavirus-specific serum IgA levels also correlate with vaccine-mediated protection from rotavirus disease, although imperfectly.[169] Virus-specific serum antibody may correlate with protection in children because it signifies previous infection without necessarily being a primary effector of protection.[142,170]

Protection by Breastfeeding

The evidence that breastfeeding protects human infants from rotavirus diarrhea is mixed, with a meta-analysis of the literature showing no significant correlation between breastfeeding and rotavirus gastroenteritis.[171] In Bangladesh hospitalized children with rotavirus diarrhea are more likely to be breastfed than patients with diarrhea from other causes, which suggests that breastfeeding prevents rotavirus gastroenteritis less effectively than it prevents gastroenteritis caused by other agents.[172] In contrast, in some clinical studies of rotavirus gastroenteritis, breastfeeding is associated with reduced frequency of vomiting, less severe dehydration, or, for infants younger than 6 months, a lower risk for hospitalization.[153,173,174]

Role of Serotype in Protection

Serotype influences but does not determine the degree of protection from challenge. Protection after natural infection appears to be somewhat more reliable against viruses of the same G type as the infecting strain.[139,159] On the other hand, cross-protection between serotypes clearly also occurs, particularly after multiple infections.[139] In controlled clinical trials, immunization with a G1P[4] monovalent live-attenuated rotavirus vaccine provides detectable but diminished protection against G2P[8] circulating strains relative to protection against strains that share a VP7 or VP4 type with the vaccine, and the degree of heterotypic protection provided by monovalent or multivalent live-attenuated rotavirus vaccines exceeds the level that can be explained on the basis of G type–specific serum neutralizing antibody titers.[175-177] This finding correlates with the primarily homotypic neutralizing antibody response to a first infection or dose of a live-attenuated vaccine and the increasingly heterotypic responses to subsequent infections or doses.[178,179]

Heterotypic Neutralization by Antibodies

Monoclonal antibodies derived from adult human intestinal B cells, although limited in number, provide insight into potential mechanisms of heterotypic protection from rotavirus disease. Human-derived heterotypic neutralizing antibodies (those that neutralize multiple P types) are largely directed at VP5*, although heterotypic neutralizing antibodies directed against VP8* (neutralizing multiple P types) and VP7* (neutralizing multiple G types) are also found.[180] These results are similar to those obtained with monoclonal antibodies derived from mouse immunization except that most identified human monoclonals that recognize VP8* bind heterotypically without neutralizing in vitro, whereas many animal monoclonals that recognize VP8* bind and neutralize homotypically.[133,134,180,181] Therefore antibodies that recognize VP5* probably make important contributions to heterotypic protection of humans against rotavirus, with some potential contribution by a subset of antibodies that recognize VP8* and VP7 as well.

Mechanisms of Neutralization by Antibodies

The mechanisms of rotavirus neutralization have been examined in detail using mouse-derived monoclonal antibodies. Neutralizing monoclonal antibodies that bind the VP8* receptor binding domain, which forms the heads of the VP4 spikes, block the attachment of neuraminidase-sensitive rotaviruses to cells or trigger virus uncoating.[182–184] A neutralizing monoclonal antibody that binds the hydrophobic tip of the β-barrel domain of the VP5* fragment of VP4 acts after cell attachment and blocks the binding of rearranging VP4 to membranes.[35,182,183] Neutralizing antibodies that bind VP7 on the virion prevent VP7 trimer dissociation and virion uncoating in low calcium conditions.[185,186] Antibodies with epitopes that span the calcium-stabilized interface between the subunits of a VP7 trimer can cross link the virion surface and neutralize as monomeric Fab; those that bind within a single VP7 molecule require divalency.[33,37]

Protection by Antibodies Against VP6 and Intracellular Neutralization

Nonneutralizing antibodies that recognize the middle-layer protein VP6, which is buried under the VP7 shell on intact virions, may contribute to heterotypic protection from rotavirus gastroenteritis by neutralizing subviral particles intracellularly.[187] IgA monoclonals that bind in the

mRNA exit channel through the VP6 layer can block transcription by DLPs in vitro. These antibodies do not neutralize intact virions in cell culture but do protect mice from rotavirus infection when secreted into the serum by "backpack" hybridoma tumors.[187–189] Host J-chains are required for heterotypic protection after immunization of mice with recombinant virus-like particles (VLPs) containing only VP2 and VP6.[190] These observations suggest that anti-VP6 IgA can block infection by binding DLPs and interfering with viral transcription while the antibody is transcytosed across the intestinal epithelium. However, protection is not provided by immunogens that lack VP4 and VP7 in all animal models. For example, immunization of gnotobiotic pigs with VP2/6 VLPs boosts protective responses elicited by immunization with live virus, but primary immunization with these VLPs does not protect gnotobiotic pigs from diarrhea on challenge with a virulent human rotavirus strain.[191] Alternatively, llama single-chain antibody fragments that lack light chains and recognize VP6 can neutralize virions across serotypes, presumably by accessing VP6 through the pores in the VP7 shell.[192]

Mouse Studies on the Roles of T-Cell–Mediated, Antibody-Mediated, and Innate Immunity in Viral Clearance and Protection From Reinfection

Studies in an adult mouse model of rotavirus infection and an infant mouse model of rotavirus diarrhea have helped to elucidate the roles of T-cell–mediated, antibody-mediated, and innate mechanisms in the control and prevention of rotavirus infection. Mice that are T-cell deficient on the basis of an αβ/γδ T-cell receptor knockout clear primary infection but somewhat later than immunocompetent mice, mount a modest IgA response (primarily against VP6), and are resistant to reinfection on challenge.[193] In contrast, most B-cell–deficient mice (J_HD knockouts) with intact $CD8^+$ T cells also clear primary rotavirus infections but can be reinfected.[194] Thus humoral immunity appears to have a central role in protecting mice from infectious challenge. Innate responses to rotavirus are essential triggers for the humoral immune response. Depletion of plasmacytoid dendritic cells or abrogation of type I IFN production from these cells in rotavirus-infected mice diminished the production of rotavirus-specific IgG and IgA in serum and rotavirus-specific IgA in stool.[195]

Although some rotavirus-inoculated adult mice with severe combined immunodeficiency disease (SCID) persistently shed virus, 40% of SCID mice on a C57BL/6 background do clear the infection despite the lack of adaptive immunity.[193] Chronic shedding of rotavirus from *RagI*$^{-/-}$ mice, which lack mature T and B cells, can be completely eliminated by administration of flagellin, independent of both an adaptive immune response and an IFN response.[196] Flagellin induces a protective gene expression program in intestinal epithelial cells by its interaction with Toll-like receptor 5 (TLR5) on dendritic cells, with subsequent production of interleukin-22 (IL-22), and also induces an inflammasome response by its interaction with nucleotide oligomerization domain (NOD)-like receptor 4, with subsequent production of IL-18.[196] The innate immune-mediated clearance of rotavirus infection by flagellin administration can be completely reproduced by administration of IL-22 and IL-18.[196] Consistent with these findings, infant mice deficient in inflammasome components are more susceptible to rotavirus diarrhea and support higher levels of rotavirus replication than wild-type infant mice.[197] Absent the administration of flagellin or IL-18, inflammasome-mediated restriction of rotavirus infection is triggered when short stretches of rotavirus dsRNA trigger the intestinal epithelial cell-specific NOD-like receptor Nlrp9b via RNA helicase Dhx9.[197] Rotavirus antagonizes these innate immune responses by several mechanisms, including NSP1-mediated degradation of transcription factors IFN regulatory factor 3 (IRF3), IRF5, and IRF7; NSP3-mediated interference with host mRNA translation; and sequestration of rotavirus dsRNA in viroplasms.[198,199]

Evidence for the Role of Innate Immunity From Studies in Human Cells

Studies in human-derived intestinal cell lines, organoids, enteroids, and peripheral blood mononuclear cells (PBMCs) confirm the essential role of innate immunity in control of rotavirus infection that is indicated by studies in mice and in nonhuman mammalian cell lines. Treatment of a human intestinal cell line (Caco2 cells) and of human intestinal organoids with exogenous IFN reduces rotavirus replication.[200] Increasing endogenous IFN expression in human enteroids by knocking down stromal antigen 2 (STAG2) expression also reduces rotavirus replication.[201] Conversely, reducing basal expression of IFNs in human intestinal cells by silencing signal transducer and activator of transcription 1 *(STAT1), STAT2,* and *IFR9* genes increases rotavirus replication.[200] Although infecting human intestinal cells with simian rotavirus (strain SA11) stimulates the expression of RNAs encoding type I and III IFNs, no expression of IFN proteins results, consistent with the host translation interference mediated by NSP1.[200] Confirming the essential role for innate immunity in stimulating an adaptive antibody response, when human PBMCs are infected in vitro with rotavirus, B-cell activation requires the production of type I IFNs by plasmacytoid dendritic cells.[195]

DIAGNOSIS

Clinical Utility of Virologic Diagnosis

Rotavirus gastroenteritis is not clearly distinguished from acute gastroenteritis due to other agents on clinical grounds alone. Because the standard treatment for rotavirus gastroenteritis is rehydration and supportive care, a specific microbiologic diagnosis is not required in most cases. However, with prolonged diarrhea, in complicated cases, in immunocompromised hosts, when alternative diagnoses are considered, or when epidemiologic or infection control data are needed, it may be desirable to establish rotavirus as the etiologic agent. Definitively diagnosing rotavirus gastroenteritis may also prevent the unnecessary and potentially harmful use of antibiotics.

Diagnosis by Antigen Detection

Rotavirus can be detected by numerous techniques, including a variety of commercial antigenic assays, RT-PCR, electron microscopy, immune electron microscopy, polyacrylamide gel electrophoresis (PAGE) for viral genomic RNA, and viral culture. Detection of viral antigen in stool or rectal swabs, most commonly using enzyme-linked immunosorbent assay (ELISA) or latex agglutination formats, forms the basis for practical, commercially available, and widely used diagnostic kits.[202] Latex agglutination is particularly suitable for use in areas with limited resources, although a confirmatory technique is desirable to evaluate indeterminate results because of the limited sensitivity of the test.[202,203] Commercial antigenic assays primarily detect the VP2 and VP6 proteins of the subviral double-layered particle and detect only group A rotaviruses. Serotype-specific ELISAs, based on recognition of VP7 or VP4, allow determination of serotype without the need to perform neutralization assays.[204] Although there are various techniques for measuring serum, fecal, and salivary antibodies against rotavirus, the acute and generally self-limited nature of rotavirus infections limits the usefulness of these techniques for clinical decision making.

Diagnosis by Reverse-Transcriptase Polymerase Chain Reaction

Multiplexed RT-PCR has become a major diagnostic technique used in epidemiologic studies. RT-PCR allows determination of P and G types and permits finer definition of strain differences.[205,206] Real-time RT-PCR provides greater sensitivity and speed than conventional or nested PCR diagnosis.[207] DNA oligonucleotide microarray methods offer greater robustness to sequence drift, which can prevent PCR amplification if a primer binding site is affected, and greater ability to distinguish a mixed infection from a single-strain infection.[208] Micromass sequencing allows metagenomic analysis of the gut microbiome, which includes rotaviruses.[209]

Diagnosis by Electron Microscopy, Electrophoresis, and Viral Culture

Both electron microscopy and PAGE can detect unusual strains of rotavirus (such as non–group A rotaviruses) that might be missed by standard antigenic or nucleic acid–based assays. Electron microscopy of stool specimens negatively stained with phosphotungstic acid is rapid and, despite only moderate sensitivity, has high specificity because of the distinctive appearance of rotavirus particles (see Fig. 150.2).[210]

Electrophoresis of simply prepared stool suspensions on polyacrylamide gels, followed by silver staining for the pattern of 11 segments of genomic dsRNA, allows both diagnosis and tracking of rotavirus strains in molecular epidemiologic investigations.[211] Rotavirus can be detected, although with relatively low sensitivity, by growth in cell culture. Human rotavirus strains have proved more difficult to culture routinely than most animal rotavirus strains, but in many cases human rotavirus strains can be propagated in MA104 cells or primary green monkey kidney cells grown in roller tubes with the addition of trypsin to the cell culture medium.[144,212]

Future Diagnostic Trends

Although still an experimental tool, direct whole-genome sequencing of rotavirus obtained directly from stool without culture has potential as part of a general trend toward increased use of sequencing in viral diagnostics. To overcome the challenges posed by the multisegment RNA rotavirus genome, high sequence variability, and inhibitors in the stool matrix, capture of virus on standard commercial rotavirus immunoassay plates has been combined with RNA extraction, reverse transcription, amplification with random primers, and next-generation sequencing to produce reliably >90% sequence coverage from stool samples containing diverse rotavirus strains.[213]

THERAPY

Overview

No specific antiviral therapy is recommended for rotavirus gastroenteritis. Because rotavirus gastroenteritis is generally self-limited, and dehydration is the primary cause of morbidity and mortality, rehydration and restoration of electrolyte balance are the primary therapies. The Federation of International Societies of Pediatric Gastroenterology, Hepatology, and Nutrition (FISPGHAN) Working Group has systematically reviewed and compared the multiple recommendations available for the treatment of pediatric gastroenteritis and developed a set of consensus universal recommendations for the management of acute diarrhea in nonmalnourished children.[214] World Health Organization (WHO) has published guidance on the treatment of acute diarrhea and dehydration in both well-nourished and malnourished children.[215,216]

Rehydration

Oral rehydration solution (ORS) is effective in treating dehydration related to rotavirus gastroenteritis, even in the presence of moderate vomiting, and is preferred over intravenous (IV) rehydration in cases of mild or moderate dehydration. The effectiveness of ORS is based on the solute-coupled cotransport of sodium by enterocytes, which continues to operate even in the damaged gut.[217] Effective solutes include glucose, amino acids, and short oligopeptides. In 2002 WHO revised the recommended standard ORS formula to a lower osmolarity (245 mOsm/L) solution, which is associated with less vomiting, lower stool output, and a reduced need for IV infusions. The low-osmolarity formulation is 75 mM sodium, 20 mM potassium, 65 mM chloride, 10 mM citrate, and 75 mM glucose (see reference for acceptable variations).[218] The lower sodium losses resulting from diarrhea due to enteric viruses than from diarrhea due to cholera or enterotoxigenic *Escherichia coli* allows lower sodium content in the oral rehydration solution.[219] In children with severe acute malnutrition and dehydration, WHO recommends an alternative oral rehydration solution, ReSoMal (see reference for formulation).[216]

In case of severe dehydration, initial IV hydration with an isotonic solution, such as Ringer's lactate or normal saline, is recommended, with oral rehydration instituted once the child can drink.[215] Failure of oral rehydration can be addressed by IV hydration or by enteral rehydration with ORS through a nasogastric tube, which is also effective and is associated with fewer side effects than IV hydration, particularly in malnourished children.[214–216]

Despite the depressed disaccharidase levels associated with rotavirus gastroenteritis, it is recommended that nursing infants continue to breastfeed during rehydration and that children resume a diet as soon as they can tolerate feeding.[214–216] The early reinstitution of an age-appropriate diet has nutritional benefits and shortens the duration of diarrhea by about half a day.[220]

Zinc Supplementation

Randomized controlled trials in developing countries have demonstrated that, for children older than 6 months, zinc supplementation during and for a short time after an episode of diarrhea can decrease the duration of diarrhea by approximately half a day on average and decrease the probability that diarrhea will last more than 7 days, but the treatment is associated with a small increase in the incidence of vomiting.[221,222] Because the benefits of zinc supplementation outweigh the risks in areas where zinc deficiency is common, WHO recommends zinc supplementation (10–14 days of 10 mg/day in children ≤6 months and 20 mg/day in children ≥6 months).[215] Zinc supplementation is not recommended in well-nourished populations, in which its benefits have not been established.[214]

Antidiarrheal Agents

Racecadotril (no commercial preparations available in the United States) is an enkephalinase inhibitor that reduces intestinal hypersecretion and has been evaluated as an adjunct to oral rehydration solutions. Although some randomized clinical trials indicate that the addition of racecadotril can decrease mean stool output in inpatients and the mean number of diarrheic stools in outpatients, two randomized, double-blind, placebo-controlled clinical trials in India showed no significant effect.[214,223] Smectite, a multilamellar aluminum-magnesium clay silicate does reduce the duration and volume of diarrhea in children, with trials in Peru and Malaysia showing a greater effect in rotavirus gastroenteritis than in nonrotavirus gastroenteritis, but it has not been demonstrated to reduce the need for IV hydration.[224,225] Although racecadotril and smectite are included in some regional guidelines, neither is recommended by the FISPGHAN Working Group.[214] Bismuth subsalicylate (Pepto-Bismol) has been shown to decrease the duration of diarrhea and the intake of oral rehydration solutions in children with gastroenteritis,[226] but the modest benefits observed, need for frequent administration, and a theoretical possibility of Reye syndrome related to salicylate absorption argue against routine use of this agent. Because of complications including ileus and respiratory depression, antimotility agents such as loperamide have no role in the treatment of childhood gastroenteritis.[214]

Antiemetics

Most antiemetics are not considered to have a favorable risk-benefit profile for self-limiting vomiting in acute pediatric gastroenteritis, although a single dose of the selective serotonin 5-hydroxytryptophan 3 receptor antagonist ondansetron may be considered in the emergency room context to enable oral rehydration (taking into account a warning for QT prolongation and cardiac arrhythmias).[214] Consistent with the role of the ENS in the pathogenesis of rotavirus gastroenteritis, a single dose of ondansetron has also been shown to reduce number of episodes of diarrhea and the duration of symptoms in pediatric gastroenteritis caused by rotavirus or norovirus.[227]

Probiotics

Evaluating the effectiveness of probiotic treatments as a supplement to ORS for rotavirus gastroenteritis is complicated by the variety of preparations available, the potential for differences in efficacy for viral and bacterial gastroenteritis, and a range of quality of the studies. The FISPGHAN Working Group concluded that selected probiotic strains, including *Lactobacillus rhamnosus* GG, *Saccharomyces boulardii*, and *Lactobacillus reuteri* DSM 17938 can be considered as a supplement to ORS.[214] However, recent, large, prospective, randomized, double-blind, placebo-controlled clinical studies of the efficacy of treating moderate-to-severe pediatric gastroenteritis with *L. rhamnosus* GG or with a combination of *L. rhamnosus* R0011 and *Lactobacillus helviticus* R0052 in the United States and Canada showed no evidence of any effect of the probiotic treatment on acute gastroenteritis signs, symptoms, or health care utilization outcomes.[228–229]

Passive Oral Immunotherapy and Immunoprophylaxis

Oral administration of immunoglobulins is not indicated for routine use but may have a role in treating chronic rotavirus diarrhea and may merit further evaluation for prophylaxis in high-risk settings, such as

immunodeficiency or severe prematurity, in which vaccination is unlikely to be effective. In case reports, feeding human serum immune globulin to children with chronic rotavirus diarrhea has been followed by resolution of diarrhea and viral shedding.[230] When given prophylactically to low-birth-weight infants, oral gamma globulin reduced the severity of diarrhea associated with neonatal rotavirus infections.[231] A potential rotavirus-specific therapy, llama single-chain antibodies that recognize VP6 and neutralize multiple rotavirus strains, when administered in a randomized, placebo-controlled trial to 6- to 24-month-old children with severe rotavirus diarrhea in Bangladesh, reduced stool output by 22.5%.[232]

IMMUNIZATION

Overview

Because lack of access to treatment is one of the major causes of childhood mortality from rotavirus, and improved sanitation has limited impact on rotavirus prevalence, prevention by immunization is a critical approach to decreasing the impact of this infection. As of 2018 two live-attenuated, oral rotavirus vaccines had been licensed in multiple countries, two more had been licensed in India and had achieved WHO prequalification, and two more had national licenses in China or Vietnam. Rotavirus immunization has led to substantial decreases in the burden of rotavirus disease in the regions where it had been implemented.

RotaShield and History of Intestinal Intussusception

The first human rotavirus vaccine, RotaShield (Wyeth Lederle Vaccines), was licensed in the United States in 1998. RotaShield was a reassortant, live-attenuated, oral vaccine containing four viruses, each with VP7 from the G types 1, 2, 3, or 4 presented on the genetic background of a simian rotavirus strain (RRV) that was attenuated for humans. In phase III trials RotaShield proved safe and highly effective against moderate-to-severe diarrhea in both developed (United States and Finland) and developing (Venezuela) countries.[177,233,234] However, in the year after launch, after more than 1 million doses had been administered, an increased incidence of intestinal intussusception (telescoping of the intestine) 3 to 14 days after administration of the first dose (odds ratio [OR], 21.7) and a smaller increase after the second dose (OR, 3.3) was detected, corresponding to a total excess risk for intussusception of approximately one case per 5000 to 10,000 children immunized.[235,236] As a result of these findings the manufacturer voluntarily withdrew the vaccine from the market.

The unknown mechanism of the temporal association between intussusception and immunization with RotaShield, combined with the failure to observe this complication in prelicensure clinical trials of RotaShield, increased the size of the phase III safety databases required for licensure of subsequent live-attenuated rotavirus vaccines to greater than 60,000 subjects each. Nevertheless, the development of two other live-attenuated rotavirus vaccines, Rotarix (GlaxoSmithKline, Research Triangle Park, NC) and RotaTeq (Merck & Co., Inc., Whitehouse Station, NJ), continued, with both vaccines achieving licensure with no intestinal intussusception observed in clinical trials.[175,176]

Rotarix

Rotarix, an oral, live-attenuated vaccine, is derived from a human rotavirus isolate (strain 89-12; P[8]G1) that was attenuated by serial passage in cell culture.[175] Because Rotarix is monovalent, breadth of coverage relies on cross-protection between serotypes, as has been observed after natural rotavirus infection.[139] In a phase III trial carried out in Finland and in middle-income Latin American populations, Rotarix proved effective (84.7%) against severe gastroenteritis. The vaccine was 87.3% effective against severe gastroenteritis caused by strains that shared the P[8] VP4 type with the vaccine and 91.8% effective against strains that shared both VP4 type and VP7 type (G1) with the vaccine.[175] Although efficacy criteria were not met for doubly mismatched G2P[4] strains in the initial phase III trial or after a second year of monitoring of a subset of infants from that trial, Rotarix achieved 85.5% protection against severe diarrhea caused by G2P[4] strains in a placebo-controlled, double-blind study in European infants.[175,237,238] Since Rotarix was first licensed in Mexico and the Dominican Republic in 2004, approvals have followed in more than 100 nations, including the European Union in February 2006 and the United States in April 2008. The Advisory Committee on Immunization Practices (ACIP) has recommended immunization with Rotarix at 2 and 4 months of age.[84]

RotaTeq

RotaTeq is a multivalent, orally administered, modified Jennerian vaccine. The five strains in RotaTeq are reassortants with a bovine rotavirus (strain WC3) background. Each of four strains has a VP7 from one of four prevalent human rotavirus G types (G1, G2, G3, or G4) combined with a VP4 from a bovine genotype, P[7]. The fifth strain has a VP7 from a bovine G type, G6, combined with a VP4 from the common human rotavirus P genotype, P[8].[239] In a phase III trial RotaTeq was highly effective (>98% protection) against severe rotavirus gastroenteritis and effective against any diarrhea caused by G1 or G2 rotaviruses (74.9% and 63.4% protection, respectively).[176] RotaTeq was licensed in the United States and the European Union in 2006 and has since been licensed in more than 90 countries. The ACIP has recommended RotaTeq for routine use in the United States, with doses given at 2, 4, and 6 months of age.[84]

Contraindications for Allergic Reactions, Severe Combined Immunodeficiency Disease, and Elevated Risk of Intestinal Intussusception

Since launch, Rotarix and RotaTeq have had good overall safety profiles, but two issues have led to the addition of new contraindications to the original contraindication of severe allergic reaction to a previous dose of rotavirus vaccine or to a vaccine component. First, the finding of prolonged shedding of vaccine virus associated with diarrhea in children with SCID led, in 2009 and 2010, to the addition of contraindications to the immunization of infants who have SCID.[240–241]

Second, although association of immunization with intestinal intussusception was not observed in clinical trials of Rotarix or RotaTeq, postlicensure surveillance in Australia, Mexico, and the United States detected temporal clustering of intestinal intussusception 3 to 7 days after administration of the vaccines, particularly after the first dose, with an excess risk of 1 to 5 cases per 100,000 infants immunized.[242–246] The Centers for Disease Control and Prevention (CDC) estimated that, if the risk exists, immunization could cause 50 to 60 excess cases of intussusception per year in the United States, while preventing more than 50,000 hospitalizations for rotavirus disease.[247] In 2011, to reduce the potential risk of vaccine-associated intussusception, a history of intestinal intussusception (Rotarix and RotaTeq) and an uncorrected congenital malformation of the GI tract that would predispose to intussusception (Rotarix) were added as contraindications to rotavirus immunization.[247] An analysis of the risk of intussusception after immunization with Rotarix in Africa found no clustering of cases in the week after the first dose.[248] The apparent lack of association between rotavirus immunization and intussusception in several lower-income African countries is reassuring, especially given the higher case fatality for intussusception in low-income countries.[248] Analysis of the RotaShield experience indicated that infants who received a first dose at ≥90 days of age contributed disproportionately to the cases of intussusception.[249] In accordance, for Rotarix and RotaTeq the ACIP recommends that the first dose of vaccine be given before 15 weeks of age[84] (see Chapter 316).

Porcine Circovirus in Vaccine Manufacturing

In March 2010 a manufacturing issue led the US Food and Drug Administration (FDA) to temporarily suspend use of Rotarix. Metagenomic analysis of a vaccine sample revealed porcine circovirus 1 (PCV-1) DNA.[250] Investigations by the manufacturer confirmed the presence of infectious PCV-1 in Rotarix cell banks, viral seeds, and vaccine lots, including those used in the large phase III safety studies.[251] PCV-1 and PCV-2 DNA fragments, but no infectious virus, were detected in RotaTeq samples, and irradiated porcine trypsin used in manufacturing was identified as the source of the PCV-2 DNA.[251,252] PCV-1 causes no known disease in animals or humans; PCV-2 is not known to cause human disease but is a cofactor for the development of pig wasting disease.[253]

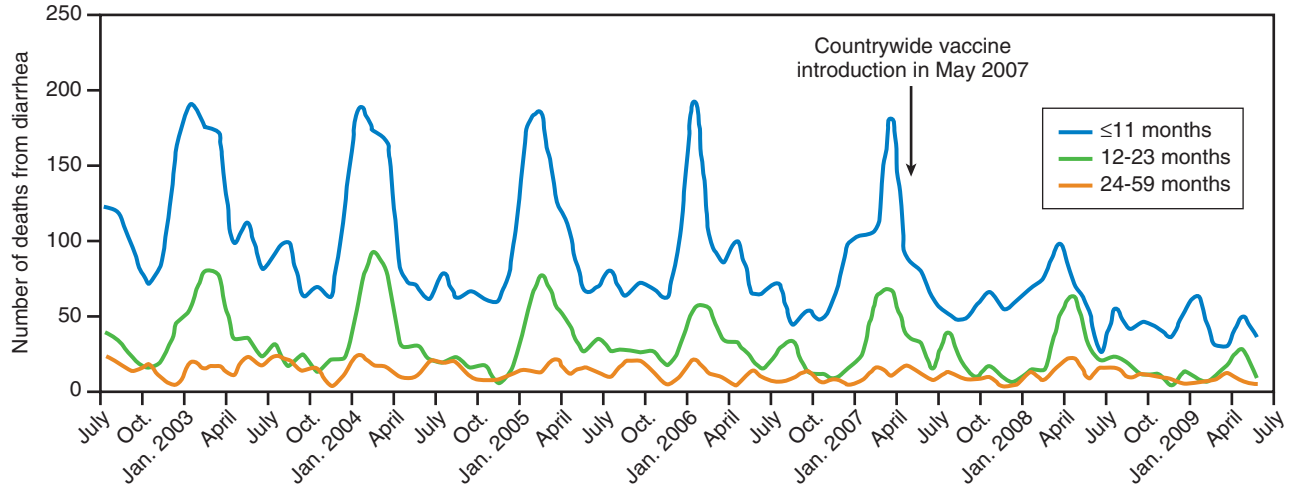

FIG. 150.4 Number of diarrhea-related deaths among children 59 months of age or younger from July 2002 through May 2009 in Mexico, according to age group. *(From Richardson V, Hernandez-Pichardo J, Quintanar-Solares M, et al. Effect of rotavirus vaccination on death from childhood diarrhea in Mexico.* N Engl J Med. *2010;362:299–305.)*

Both PCV-1 and PCV-2 are commonly present in pork products and are often ingested by humans with no known health consequences.[251] In May 2010 the FDA concluded that the benefits of immunization with Rotarix and RotaTeq outweigh any theoretical risks based on the PCV findings, that immunization should continue, and that manufacturers should develop PCV-free vaccine preparations.[254]

Reduction in Rotavirus Disease Burden Resulting From Immunization

Rotarix and RotaTeq have had a major impact on hospitalizations due to rotavirus infection and gastroenteritis overall in the regions where they are widely distributed (see "Epidemiology" earlier). The estimated decrease of 55,000 acute gastroenteritis hospitalizations in the United States in 2008, shortly after vaccine introduction, corresponds to the elimination of 1 of every 20 hospitalizations among children younger than 5 years.[255] In the United States the threshold level of detection of rotavirus infections that would signal the start of the rotavirus season was never achieved on a nationwide basis in 2011–12.[256] In the decade since vaccine introduction (2006–17), with 71% to 75% vaccine coverage, effectiveness against rotavirus-associated hospitalization and emergency department visits in the United States has been 84% for RotaTeq and 83% for Rotarix.[148] In El Salvador, a low- to middle-income country where Rotarix was introduced, rotavirus hospitalization rates declined by 69% to 81% in 2008–09, which were years with vaccine coverage rates of 50% to 61%, and overall diarrhea-related health care visits during rotavirus season declined by 35% to 48% for children younger than 5 years.[257] In Mexico in 2008, at a first-dose Rotarix coverage rate of 74%, overall diarrheal deaths among children 59 months of age or younger decreased by 35%, and the reductions were sustained into 2009 (Fig. 150.4).[258] Studies in the United States, Australia, El Salvador, and Mexico have demonstrated decreases in rotavirus-associated disease in children outside the age range of rotavirus immunization programs, indicating substantial herd immunity.[148,255,257–259] Although dominant rotavirus strains have changed in the years since the introduction of the vaccines, effectiveness data indicate that both the monovalent and pentavalent vaccines provide substantial heterotypic protection, and there is no clear, sustained pattern of strain change that would allow a definitive conclusion that virus escape from the vaccine is driving the changes.[138,260]

Reduced Vaccine Efficacy in Low-Income Settings

Live-attenuated rotavirus vaccines have shown lower efficacy (typically ≤50%) against severe gastroenteritis in low-income settings than in middle-income settings (typically ≈75%) or in high-income settings (typically >85%).[b] No definitive cause for the disparity has been established. Hypotheses include the effects in low-income settings of overall nutritional deficiency, micronutrient deficiency, inhibition by high breast milk antibody levels of live-attenuated vaccine take, higher levels of transplacentally transferred antirotavirus IgG, a higher burden of other enteric infections, and more frequent and larger infectious challenges.[265,266] An emerging literature suggests that differences in the intestinal microbiome may affect rotavirus vaccine take, although geographic variation in microbiome composition and the instability of the microbiome before 2 years of age result in a high degree of variability between studies.[266] A similar trend of decreased vaccine performance in resource-poor settings is observed for the live oral polio vaccine (OPV), and, although coadministered rotavirus vaccines do not decrease the immunogenicity of OPV, coadministered OPV may decrease the immunogenicity of live-attenuated rotavirus vaccines.[267]

Benefits of Rotavirus Immunization in Low-Income Settings

Despite these challenges, the greatest benefits of rotavirus immunization are actually realized in low-income settings, where the burden of rotavirus disease is greatest. Postlicensure observational analysis of the impact of rotavirus immunization on children younger than 5 years in countries with high baseline rotavirus-associated mortality showed a 60% decrease in hospitalizations and emergency department visits due to rotavirus-associated gastroenteritis and a 36% decrease in mortality due to acute gastroenteritis regardless of the causative agent.[268]

Global Rotavirus Vaccine Introduction

Because rotavirus mortality is concentrated in impoverished settings, in 2009 the Strategic Advisory Group of Experts (SAGE) on Immunization of WHO recommended the inclusion of rotavirus vaccination in all national immunization programs, strongly recommending introduction of rotavirus immunization in countries in which diarrheal deaths account for 10% or more of the mortality of children younger than 5 years.[269] Initiatives to increase global rotavirus vaccine coverage involve the Global Alliance for Vaccines and Immunization (GAVI), the Bill and Melinda Gates Foundation, the Program for Appropriate Technology in Health (PATH), the CDC, and WHO, among other organizations. As of December 2016, of the 194 countries of the world, rotavirus immunization had been incorporated into the national immunization programs in 84, in which 31% of the world's children reside[270] (see Chapter 316). Efforts to introduce the vaccines have focused heavily on Africa, owing to the >80% of rotavirus infant deaths occurring in that region.[1,270]

[b]References 175, 176, 237, 238, 257, 261–264.

Regional Rotavirus Vaccines: Lanzhou Lamb Rotavirus Vaccine, Rotavac, Rotasiil, and Rotavin-M1

In addition to Rotarix and RotaTeq, which have been introduced in global markets, there are several live-attenuated rotavirus vaccines produced in emerging markets for local use. One of these, the Lanzhou lamb rotavirus vaccine, is a live-attenuated, oral, monovalent vaccine based on an ovine rotavirus (P[12]G10) strain. It was licensed in China in 2000 without having been tested in a prelicensure efficacy trial, and more than 30 million doses have been distributed. Although observational studies have been done, comparisons to other licensed rotavirus vaccines are difficult to make for methodologic reasons, including an unusual immunization schedule, with one dose given every year for 3 years between the ages of 2 and 35 months.[271,272]

Rotavac, a human neonatal strain 116E, oral, live-attenuated monovalent vaccine candidate, has been developed by Bharat Biotech International in Hyderabad, India in partnership with a consortium of international governmental, nongovernmental, and academic institutions and with a price commitment for the public sector at the time of launch of less than US$ 1.00 per dose.[273] Rotavac provides an example of the potential for rotavirus vaccines developed and manufactured in lower-income settings to be more affordable, which could enable increased, sustainable vaccine coverage in regions of high rotavirus mortality. The vaccine virus is a naturally occurring G9P[11] reassortant, with a bovine genome segment encoding VP4 on a background of human rotavirus genome segments, and it generally caused asymptomatic infection, even before adaptation to growth in cell culture.[273] The vaccine showed 53.6% efficacy against severe rotavirus gastroenteritis during the first year of life in a randomized, double-blind, placebo-controlled, multicenter phase III trial in India.[273] The vaccine was licensed in India in 2014 and prequalified by the WHO in 2018, enabling distribution beyond India by United Nations agencies.[274] A staged roll-out of the vaccine in India is underway.

Rotasiil, a pentavalent (G1, G2, G3, G4, G9) bovine-human reassortant, oral, live-attenuated vaccine developed by the Serum Institute of Pune, India, based on strains from the US National Institutes of Health, has been tested in Niger, where it showed 66.7% efficacy against severe rotavirus gastroenteritis and in India, where it showed 36% efficacy.[275,276] Rotasiil is heat stable, not requiring a cold chain for terminal distribution and administration. The vaccine was licensed in India in 2017 and prequalified by WHO in 2018.

Rotavin-M1, a monovalent G1P[8] live-attenuated vaccine manufactured by the Center for Research and Production of Vaccines and Biologicals (Hanoi, Vietnam), is based on a strain isolated from a Vietnamese child and was licensed in Vietnam in 2012 based on immunogenicity and safety data.[8,277] Additional live-attenuated vaccine candidates are in development.

Nonreplicating Rotavirus Vaccine Candidates

New approaches to rotavirus vaccines, including nonreplicating rotavirus vaccine candidates, could provide greater immunogenicity in impoverished settings, easier combination with other routine childhood vaccines, and a definitive solution to concerns over intestinal intussusception.[278] Such candidates include inactivated rotavirus particles[279]; recombinant noninfectious virus-like particles[280]; and recombinant constructs in which the stable and easily expressed VP8* lectin domain is mounted on multivalent display platforms to increase its immunogenicity.[281,282] A subunit vaccine candidate in which a P[8] VP8* receptor–binding domain is fused to the P2 epitope of tetanus toxin and adsorbed to aluminum hydroxide has advanced to safety and immunogenicity testing in human toddlers and infants.[283] Such nonreplicating rotavirus vaccines could complement live-attenuated rotavirus vaccines, much as inactivated polio vaccines complement OPV, to lower practical barriers to universal infant immunization in the most challenging settings.

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151 Alphaviruses (Chikungunya, Eastern Equine Encephalitis)

Lewis Markoff

SHORT VIEW SUMMARY

Definition

- Alphaviruses constitute a genus of at least 29 viruses in the family Togaviridae. Alphaviruses are lipid-enveloped, and the genome is a positive-sense RNA approximately 12 kb in length.
- All human pathogenic alphaviruses are mosquito-borne.

Epidemiology

- Mosquitoes and a human or animal reservoir are required for the virus life cycle and for disease outbreak. Human-to-human transmission does not occur.
- New World alphaviruses include Eastern, Western, and Venezuelan equine encephalitis viruses (EEEV, WEEV, and VEEV, respectively), which are found in North and South America.
- Old World alphaviruses, especially Sindbis, Ross River, and O'nyong-nyong viruses, found in Europe, Africa, and Asia, cause a fever/arthralgia/rash syndrome. Chikungunya virus (CHIKV) originated in Africa and is grouped with Old World viruses, but is now found in North and South America, as well as in Africa and Asia.

Pathogenesis

- Alphaviruses enter cells by receptor-mediated endocytosis and exit by budding from the plasma membrane.
- After infection via mosquito bite, alphaviruses initially replicate in various cell types in the skin, resulting in viremia. Viremia permits invasion of the central nervous system (CNS) by alphaviruses that cause encephalitis or invasion of the joints and internal organs by viruses that cause fever, arthralgia, and rash. Infection may rarely be acquired via transfusion of infected blood or by aerosol.
- Alphaviruses suppress the innate immune response, primarily by inhibiting JAK/STAT signaling.
- In the encephalitis caused by New World alphaviruses and in the arthralgia caused by Old World alphaviruses, disease pathology is the result of the cell-mediated immune response to the infection.
- Recovery from the disease state is mediated by the combined effects of virus-neutralizing antibodies and cytotoxic T cells.
- In the CNS, virus replication in neurons may be suppressed indefinitely by antibody-secreting B cells.

Diagnosis

- Knowledge of the patient's travel history is of major importance in diagnosis.
- The fever/arthralgia/rash syndrome caused by Old World alphaviruses can be confused with many other viral exanthems.
- Alphavirus encephalitis is similar in clinical presentation to that caused by the flaviviruses West Nile virus and St. Louis encephalitis virus.
- Culture of virus from blood or detection by reverse transcriptase–polymerase chain reaction has a low sensitivity for diagnosis of alphavirus encephalitis but may occasionally yield positive results in the acute phase of fever/arthralgia/rash syndrome.
- Assays to detect virus-neutralizing antibodies in blood may be diagnostic in the late acute phase or the convalescent phase of disease.

Therapy

- At present, there are no products licensed for treatment.
- For encephalitis, supportive measures and intensive nursing care are currently indicated.
- For fever/arthralgia/rash syndrome, analgesics and nonsteroidal antiinflammatory drugs are the main treatment options.

Prevention

- Veterinary vaccines are available for horses, and their immunization against WEE, EEE, and VEE is required in the United States.
- There are no licensed vaccines for prevention of alphavirus diseases in humans, but unlicensed vaccines against VEE, EEE, WEE, and CHIK are available for at-risk laboratory workers.
- Vaccines are under development, particularly against CHIK fever.
- Disease prevention is best effected by mosquito eradication programs, avoidance of mosquito-infested areas, and use of protection from mosquito bites in locations where exposure to possibly infected mosquitoes is unavoidable.

Alphaviruses constitute a genus in the Togaviridae family of enveloped RNA viruses.[1] All the medically important alphaviruses are mosquito vector borne. In nearly all instances, the life cycles of alphaviruses require specific mosquito vectors and nonhuman mammal or bird species. Thus humans are innocent bystanders in virus spread. Three mosquito-borne alphaviruses have historically been associated with human disease in the United States: eastern equine encephalitis (EEE), western equine encephalitis (WEE), and Venezuelan equine encephalitis (VEE) viruses. However, the incidence rates of WEE and VEE are nil to low, respectively, thus far in the 21st century. EEE virus (EEEV), WEE virus (WEEV), and VEE virus (VEEV) are "New World" alphaviruses, defined by their antigenic and nucleotide sequence relatedness, as well as by their geographic distribution. "Old World" alphavirus species of major importance include chikungunya (CHIK) (in Africa, Asia, and tropical North and South America); O'nyong-nyong (Africa); Mayaro (South America); Ross River (Australia, Oceania); Sindbis (Africa, Scandinavia, the countries of the former Soviet Union, Asia); and Barmah Forest (Australia) viruses. The Old World alphaviruses primarily cause fever, rash, and arthropathy. Relevant information on medically important alphaviruses and some related species is presented in Table 151.1.

HISTORY

WEEV and EEEV were initially recovered from the brains of horses with encephalitis in California (1930) and New Jersey (1933), respectively. By 1938, both of these agents had been established as causes of encephalitis in humans.[2] Similarly, VEEV was first isolated from the brains of horses in Venezuela, during an epidemic of encephalitis in 1938.[3] The first reported cases of VEE in humans were attributable to aerosol spread of infectious virus from equine pathology specimens to laboratory personnel, in 1943. Naturally acquired VEE in humans was first reported from Colombia in 1952, in association with an epizootic disease in equines.[4] The first reports of human cases of VEE in the United States

TABLE 151.1 Medically Important Alphaviruses and Some Related Alphavirus Species

ANTIGENIC COMPLEX	VIRUS	GEOGRAPHIC DISTRIBUTION	ANIMAL RESERVOIR	HUMAN VECTOR[a]	HUMAN DISEASE (ANIMALS AFFECTED)
EEE	EEE	NA, SA, Caribbean	Birds	*Aedes*	Encephalitis (horses, birds)
WEE	WEE	NA, SA	Birds, horses	*Culex tarsalis*	Fever, encephalitis (horses, birds [especially emus])
	Aura	SA	Birds		
	Fort Morgan	Colorado	Birds		
	Highlands J	Eastern United States	Birds	*Culex, Aedes*	Encephalitis (turkeys, pheasants, partridges, ducks, emus, horses)
	Kyzylagach	Azerbaijan	Birds		
	Sindbis	AUS, AFR, EUR, Asia Minor	Birds	*Aedes*	Fever, arthralgia, rash
	Whataroa	AUS, NZ	Birds		
VEE	VEE	NA, SA	Horses and others	*Psorophora, Aedes*	Fever, encephalitis (horses)
	Cabassou	SA			
	Everglades	Florida	Mammals	*Ochlerotatus*	Encephalitis
	Pixuna	Brazil	Mammals		
SF	Semliki Forest	AFR	Mammals	*Aedes*	Fever, arthralgia, rash? (rare)
	Bebaru	Asia			
	Chikungunya	AFR, Southeast Asia, Philippines	Primates	*Culex, Aedes*	Fever, arthralgia, rash
	Getah	Asia	Mammals	*Culex, Aedes*	Fever? (horses)
	Mayaro	SA		*Haemagogus, Aedes*	Fever, arthralgia, rash
	O'nyong-nyong	AFR		*Anopheles*	Fever, arthralgia, rash
	Ross River	AUS, South Pacific	Mammals	*Aedes, Culex*	Fever, arthralgia, rash
BF	Barmah Forest	AUS	Birds	*Aedes*	Fever, arthralgia, rash

[a]The mosquito vector genus or species required for epizootic transmission of infection is shown. No epizootic vector is listed for viruses that rarely cause disease or for which there are no reports of disease.
AFR, Africa; *AUS,* Australia; *BF,* Barmah Forest; *EEE,* eastern equine encephalitis; *EUR,* Europe; *NA,* North America; *NZ,* New Zealand; *SA,* South America; *SF,* Semliki Forest; *VEE,* Venezuelan equine encephalitis; *WEE,* western equine encephalitis.

were published in 1968.[5] Retrospective analysis of historical accounts suggests that CHIK virus (CHIKV) caused epidemics of fever, rash, and arthralgia in Indonesia (1779); East Africa (1823, 1870); India (1824, 1871, 1901, 1923); the Far East (1901); West Africa (1925); and the southeastern United States (1827). The virus was first isolated during an epidemic in Tanzania in 1952 and 1953.[1] An epidemic of CHIK fever that originated in Africa in 2004 and then spread to islands in the Indian Ocean, India, and elsewhere in South Asia, Africa, Europe, and tropical areas of North and South America[6] is on the wane in at least some of these areas as of 2017.

MECHANISM OF REPLICATION

Alphaviruses are lipid-enveloped RNA viruses with a diameter of 60 to 70 nm. The genome is a capped, 11- to 12-kilobase, bicistronic positive-strand (or message sense) RNA. In virus particles, genome RNA is tightly associated with the virus-coded core protein, forming an ordered icosahedral nucleocapsid structure. Each virus particle consists of one nucleocapsid surrounded by the lipid envelope. Two envelope glycoproteins, E1 and E2, are inserted in the lipid membrane as heterodimers. Trimers of heterodimers of E1 and E2 form 80 "spikes" that are distributed regularly on the outer membrane surface.[7] Two additional small structural proteins, the 6K protein and the TF protein, share the same open reading frame and are also associated with membranes. 6K protein enhances membrane permeability by inducing pore formation and induces caspase-dependent apoptosis. TF is differentiated from 6K by ribosomal frame shifting within the C-terminus of the 6K protein coding sequence.[8] Palmitoylation of the TF protein is required for its incorporation into viral membranes, and abrogation of either TF synthesis or its palmitoylation inhibits virion morphogenesis.[9] E2 appears primarily responsible for attachment of virus to the cell surface. E2 antibodies, but not generally E1 antibodies, can neutralize virus infectivity. E1 contains fusion and hemagglutinin activities.[1]

Alphaviruses enter cells primarily by receptor-mediated endocytosis. Several different receptors have been identified for different alphaviruses in different cell types in vitro. Two such receptors are found on dendritic cells: DC-SIGN (*d*endritic *c*ell–specific *i*ntercellular adhesion molecule-3-*g*rabbing *n*onintegrin) and L-SIGN (*l*iver/*l*ymph node–specific *i*ntercellular adhesion molecule-3-*g*rabbing *n*onintegrin).[10] Their affinity for dendritic cells and other cell types central to the host immune response has much to do with ongoing interest in alphaviruses as vaccine vectors for foreign antigens. After the E2 envelope protein binds to the cellular receptor, the virus particle is engulfed in a clathrin-coated endocytic vesicle that bears markers of an "early endosome." Subsequent acidification of the endosome triggers disassembly of the E1-E2 heterodimer and a low-pH–dependent fusion reaction with the endosomal membrane, mediated by E1. Fusion of the virus lipid envelope with the membrane of the endosome ultimately results in the release of virus RNA into the cytoplasm. Among the different species of alphaviruses, there is some variation on this theme. Some alphaviruses traverse the early endosomal pathway to fuse with the membrane of late endosomes. Some older and some recent data suggest that Sindbis virus (SINV) (for one example) may bypass the endocytic pathway entirely and enter cells by direct fusion with the plasma membrane.[11]

Once virus RNA has entered the cytoplasm, the four viral nonstructural proteins (nsp1-4) are then derived by direct translation of the 5′-terminal two-thirds of genomic RNA and sequential cleavage of the resulting polyprotein. Nsps 1 through 3 contain various activities necessary for capping of viral RNAs (nsp1), protease cleavage of the polyprotein (nsp2), and accessory functions required for RNA synthesis (nsp2 and nsp3). Nsp4 contains RNA-dependent RNA polymerase activity. Partial cleavage products of the nonstructural polyprotein (P123 + nsp4) initiate negative-strand RNA synthesis after infection. Eventually, nsp1, uncleaved P23, and nsp4 form a replication complex in virus-induced cytoplasmic vacuoles that is active in both negative-strand and genome RNA synthesis. Finally, cleavage of P23 results in a complete switch from negative-strand RNA synthesis to synthesis of new positive-sense genomes exclusively.[1] Cleavage of P23 is also required to release nsp2, which mediates evasion of the cellular innate immune response.[12] Viral structural proteins are encoded by a subgenomic (26S) messenger RNA collinear with the 3′-terminal one-third of the genome.

Initial products of translation of the 26S messenger RNA include a 62-kDa E2 precursor polypeptide, PE2 or "p62"; E1; and the hydrophobic 6K and TF membrane proteins. PE2 and E1 glycoproteins form a heterodimer in the endoplasmic reticulum that is transported to the plasma membrane through the Golgi secretory pathway. PE2 is cleaved by cellular enzymes during exocytosis to produce E2, and the E2/E1

heterodimer is acquired in *trans* by nascent virus particles via budding of nucleocapsids at the plasma membrane.[13] Thus the lipid envelope in mature particles is derived from the plasma membrane of the cell. The cleavage of PE2 destabilizes the heterodimer with E1 such that it is more readily dissociated by low pH, which, as noted, activates the capacity of mature virions for fusion.[1]

Phylogeny

The alphavirus genus contains at least 29 different viruses classified into seven serocomplexes.[14] EEE, VEE, and WEE are prototype viruses for each of three antigenic complexes. The WEE genome and those of WEE-like viruses, except for Aura virus (see later), are intragenic recombinants: the nonstructural and core protein genes are derived from an EEE-like ancestral genome, whereas the structural glycoproteins E1 and E2 are derived from the genome of a Sindbis-like virus.[15,16] Because the serologic basis for speciation is largely dependent on antigenicity of E2 in the hemagglutination inhibition (HI) test, some of the Old World alphaviruses—SINV and Sindbis-like viruses—group with WEE complex viruses (see Table 151.1). This is also true for a phylogenetic analysis based only on sequences of the viral structural proteins.[16] CHIKV, O'nyong-nyong virus, Mayaro virus, and Ross River virus (RRV) are grouped with Semliki Forest virus in a fourth complex. Three additional alphavirus serogroups contain only a single virus species: Barmah Forest virus (BFV) (see Table 151.1) and Middelburg and Nduma viruses (not shown in Table 151.1).[14,16]

A phylogenetic study of alphaviruses, derived from a Bayesian analysis of whole-genome sequences, is in essential agreement with their serologic classification, although the resolution is slightly different.[17] All the medically most important alphaviruses group into two clades: the Semliki Forest clade, including CHIKV, O'nyong-nyong virus, and RRV; and the Sindbis–equine encephalitis clade, divided into three complexes including major members SINV, VEEV, WEEV, and EEEV. This analysis also suggested that alphaviruses arose from a primordial aquatic virus ancestor. Viruses that share medically important characteristics, in addition to their genetic and antigenic relatedness, are widely dispersed geographically. For example, the geographic distribution of the EEE complex viruses is quite different from that of the VEE complex viruses, yet members of these complexes share encephalitic potential in equines and humans. Similarly, viruses of the Semliki Forest virus complex, Mayaro (limited to Latin America), and O'nyong-nyong (limited to Africa) produce identical fever/arthralgia/rash syndromes.

EPIDEMIOLOGY

Encephalitis-Causing Alphaviruses

Eastern Equine Encephalitis Viruses

Alphaviruses are limited in their geographic distribution by the range of their respective arthropod vectors. EEEV infection occurs focally along the eastern and Gulf coasts of the United States, with a very few human cases reported also in central states that border the Mississippi River. Documented cases have occurred as far north as southern Canada and as far south as northern areas of South America and the Caribbean.[18] The EEE complex consists of four antigenically and genetically distinct lineages: one that circulates in North America and the Caribbean (NA) and three that circulate in Central and South America (SA).[18–20] One recent study of NA and SA EEEV isolates, based on nucleotide sequencing,[21,22] suggested that the NA and SA viruses are highly diverged and should be considered different species. Thus it is not surprising that NA and SA EEEVs differ radically in pathogenicity and ecology.

In North America, EEE is a summertime disease, occurring most frequently in children and in the elderly. Between 1965 and 2016 the maximum number of cases reported in any one year was 15, in 2012. However, the years between 2003 and 2016 were notable for both a trend toward an increase in the average number of cases per season and a slight increase in the ratio and number of cases reported in northern states compared to southern. These observations were attributed to the effects of climate change on vector spread and viability over winters.[19] From 2007 to 2016, 68 cases of neuroinvasive disease caused by EEEV were reported, typically during summer months.[20,23] Of these, 29 were reported in New England states or New York State. Although relatively rare, an outbreak is usually noteworthy because of the high case-fatality rate (50% to 70%). The incidence of disease in equids and swine (which constitute a second animal host) greatly exceeds that in humans, and outbreaks resulting in the deaths of hundreds of horses have been reported in the northeastern United States and especially Florida, among southern states. For the period 2007–2016, the US Department of Agriculture reported a total of 1724 cases of EEE in horses in the United States, with 118 cases in 2016 alone.[24] In horses, EEE can cause necrotizing infections involving multiple organ systems, including the heart, spleen, urinary tract, and gastrointestinal tract, as well as the entire central nervous system (CNS).[25]

In North America, the principal enzootic vector for EEE is the mosquito *Culiseta melanura,* which breeds in freshwater swamps and feeds on passerine birds. Infection of avian species may result in death in some cases and may be without apparent consequence in others. In either case, it results in viremia of sufficient magnitude and frequency to maintain a reservoir of infected mosquitoes.[19,26] *Culiseta melanura* has not been implicated in transmission of infection from birds to horses and humans, because this species is highly ornithophilic. However, this possibility cannot be ruled out because of the high population density of infected *C. melanura* mosquitoes during an enzootic cycle. Other possible bridge vectors include *Aedes* and *Coquillettidia* spp.[27] Horses and humans develop only low or undetectable levels of viremia and do not serve as reservoirs for further virus spread. Among birds, infection seems to be spread by the oral route as well as by mosquito bite.[22]

In summary, conditions for EEE epizootics include the presence of *C. melanura* and susceptible bird populations coincident with vector mosquitoes capable of feeding on both birds and the horses or humans in the vicinity. In temperate climates, maintenance of epizootics is theoretically interdicted by winter. However, EEEV can be isolated from the same endemic foci in successive years, and genome sequence data from several isolates suggest that the virus does persist or "overwinter" in cold climates and that EEEV strains migrate from south to north along the East Coast of the United States.[26] EEEV is highly infectious by the aerosol route.[28] However, this mode of infection poses a risk associated only with handling of infected birds or with laboratory exposure.

EEE is a rare disease in South America, with only two recent case reports.[29] Serologic studies of native populations exposed to EEEV showed that infections occur without subsequent illness, suggesting that South American strains are relatively avirulent in humans.[19] This difference in virulence between North and South American isolates may be due to differences in replication efficiency in neuronal cells and in interferon (IFN) sensitivity and is probably multigenic in origin.[19,30]

Western Equine Encephalitis Viruses

The WEE complex viruses—New World viruses WEE, Buggy Creek, Highlands J, Fort Morgan, and Aura, and Old World viruses SINV and the Sindbis subtypes—cause either arthralgic (SINV-like subgroup) or encephalitic (WEE and Highlands J virus subgroup) syndromes. Their bimodal disease potential probably reflects the relative genetic contributions to tissue tropism of the EEE-like ancestral virus core and nonstructural genes versus that of the Sindbis-like *E1* and *E2* genes, in each particular genome. WEEV is distributed primarily in the Americas. A subtype of WEE, isolated in Argentina, is presumed to be representative of endemic strains in South America.[31] In North America, WEE is a summertime disease of horses and humans in states west of the Mississippi River and in corresponding Canadian provinces. The vector is *Culex tarsalis.* Risk factors for infection include rural residence, outdoor employment in farming (because the vector favors irrigated areas), and male sex. During an epidemic, a high percentage of the adult population seroconverts, but the case-to-infection ratio ranges from less than 1:1000 in older adults to nearly unity in infants. Thus encephalitis is most frequent in infants younger than 1 year. However, encephalitis is most severe in older adults. The case-fatality rate is 3% to 4%. In contrast, EEE infection rates are low in an epidemic, but the case-to-infection ratio is higher than that for WEE and is highest in the young.[31] The near total absence of WEE case reports in the past 15 years may reflect the predominance of strains that are relatively avirulent in humans. Using a mouse model for WEE, eight different strains of WEEV displayed either high- or low-virulence phenotypes. These strains segregated into one of two genotypes that correlated with their pathogenicity.[32]

Between 1964 and 1998, 639 confirmed cases of WEE were reported to the Centers for Disease Control and Prevention (CDC). WEE cases occurred most commonly in Colorado (173 cases), Texas, North Dakota, and California, with additional significant numbers of cases in Missouri, South Dakota, and Kansas.[20] The fact that there were no new cases of WEE reported to the CDC between 1998 and 2016 suggests that control efforts and other natural variables, such as strain virulence, have had a dramatic negative effect on disease incidence. (A single case of probable WEE was reported from Uruguay in 2009.[34]) In contrast, EEE is currently estimated to cause about 1% of all cases of viral encephalitis in the United States.[19,20,35] Formalin-inactivated whole-virus vaccines against EEE and WEE are available for equines, and vaccination is currently recommended for all horses residing in North America as core prophylaxis. There are no licensed vaccines for humans.

Venezuelan Equine Encephalitis Viruses

Six antigenic subtypes of VEE are recognized. Subtype I virus strains, or variants AB, C, D, and E, are found in tropical America and are medically most important. Variants IAB and IC were associated with equine epizootics and human epidemics that occurred between the 1920s and the early 1970s. Variants ID and IE and subtype II (also known as Everglades virus) are less virulent and are associated with enzootic disease.[18] The complex genetic relationships among VEE complex viruses were recently reviewed.[36] A live virus vaccine derived from a 1AB subtype virus is thought to have been the cause of an outbreak of human disease after a 1943 vaccination campaign.

VEEV outbreaks in South America and Central America have been associated with tens of thousands of both equine and human cases.[22] For epizootic viruses, equines play an important role in maintenance because they develop high-titer viremia and are thus likely to transmit infection to mosquitoes. At least 10 mosquito species, including *Culex, Aedes, Mansonia, Psorophora,* and *Deinocerites,* have been identified as probable epidemic vectors. Epizootics have been documented in Mexico, Venezuela, Colombia, Ecuador, and Peru at intervals of 10 years or less since the 1930s. Typically, epizootics begin in areas of tropical forest during the rainy season. In the center of an epizootic, transmission usually continues until all horses are dead or immune. Spread may be to contiguous areas or may be sporadic. Major VEE outbreaks occurred in 1962, 1973, and 1995, the last outbreak involving as many as 100,000 human cases.[37,38] Nearly all viruses isolated during the 1995 outbreak were closely related to the 1C subtype viruses that had been isolated in 1973. Five years later, viruses isolated from a few scattered cases of VEE in Venezuela were related to those isolated in 1995, yet epizootic vectors were not plentiful. This suggested that epizootic viruses could persist in nature via a cryptic transmission cycle.[39] For example, a study of VEE endemicity among horses, cattle, dogs, and humans in the Gulf Coast region of Mexico during the period 2003–2010 demonstrated that the 1E subtype of VEEV circulating in that area is phylogenetically related to the 1E virus that caused a major outbreak in 1969–1971, although in a separate clade.[40]

Increased incidence of human disease typically follows outbreaks of equine disease by 1 to 2 weeks. Severe human disease with encephalitis is most common in children. Like horses, humans also develop a viremia of sufficient magnitude to infect mosquitoes. However, humans have never been implicated in epidemic transmission. Similarly, although VEE can be isolated from throat washings and is certainly infectious by the aerosol route, person-to-person transmission has not been documented.[41]

Studies comparing epizootic strains with commonly isolated enzootic ones originally suggested that these sets of VEE subtypes were highly unrelated. In fact, *Culex taeniopus,* the common vector for enzootic strains of VEE, is refractory to oral infection with epizootic virus.[42] However, comparisons of the nucleotide sequences of newly emerging (1C-like) epizootic viruses in South America suggest that they evolved from a group of enzootic 1D-like viruses by a spontaneous mutation or mutations within the *E2* gene sequence that increase the positive charge of the E2 ectodomain. A variety of additional observations implicate similar net positive charge in E2 as a major determinant of equine and human virulence.[43–45] Fig. 151.1 illustrates alternative general mechanisms by which viruses such as EEEV, WEEV, and VEEV might transition from an enzootic to an epizootic cycle.

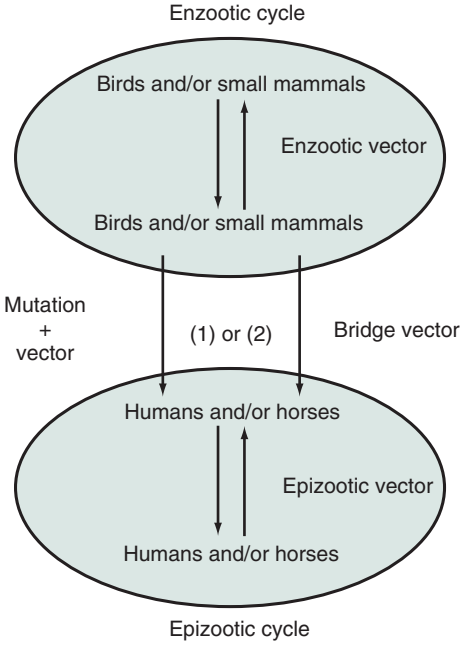

FIG. 151.1 Two different mechanisms leading to epizootic spread of alphavirus infections. The normal enzootic life cycle depends on the habits of mosquito vectors that are adapted to feed on the required small animal hosts, including birds and rodents. Virus infection spreads to horses or humans, or both, when either *(1)* a mutation occurs in the viral genome, adapting it to replication in large animals and in vector mosquitoes that are adapted to feed on them; or *(2)* a "bridging" vector transmits virus directly from animals involved in the enzootic cycle to humans or horses. Once the epizootic cycle is initiated, virus is spread by a different vector or set of vectors adapted to the large animal host. Venezuelan equine encephalitis virus is an example of an alphavirus that requires a mutation to initiate epizootic spread (mechanism 1). Eastern equine encephalitis virus is an example of an alphavirus that can spread to humans and horses by the action of a bridging vector mosquito (mechanism 2).

Horses are not amplifying hosts for enzootic strains of VEE. These viruses are principally maintained by their mosquito vector and rodents that thrive in tropical and subtropical swamps and forests. Humans living in these areas manifest a high prevalence of antibody. These viruses cause an illness resembling dengue fever and cause encephalitis sporadically, in Mexico and Central America (subtypes ID and IE) and Florida (subtype II).[42]

Alphaviruses Causing Fever, Polyarthritis, and Rash

Chikungunya Virus

Chikungunya means "to walk bent over," in reference to the crippling arthritic manifestations of the disease. *Aedes* mosquitoes of the subgenus *Stegomyia* are the principal vectors in Africa, and the virus seems to be maintained by transmission to nonhuman primates. Humans with high concentrations of virus in blood also provide a reservoir for the infection of mosquitoes. This permits epidemic spread even in urban settings, depending only on the presence of competent mosquito vectors. There are four known chikungunya virus (CHIKV) genotypes. These are the East-Central-South African, the West African, and the Indian Ocean lineages and the Asian genotype. The East-Central-South African and West African genotypes are mostly enzootic in Africa. The other two genotypes are found in Southeast Asia and India. The Asian genotype is implicated in the spread of disease to the New World.[46]

A major epidemic of CHIKV apparently started in Kenya in 2004, causing an estimated 500,000 cases in Africa, and spread initially in 2006 to the island of Réunion in the Indian Ocean. On Réunion, approximately 265,000 of 770,000 inhabitants experienced CHIK fever by the end of 2006, with 237 deaths.[47] After 2006, the outbreak spread to the southeast coast of India, the islands of Mauritius, Seychelles, and

Mayotte, and to Madagascar. At least 1.4 million cases of CHIK fever were reported in 2006–2007 in India. From 2006 at least up to 2016, the CHIK fever epidemic continued to spread into northern India, involving also neighboring areas of China in 2010[48] and countries of the Far East more distant from the epicenter. The causative strain was shown to be genetically related to the virus that initiated the epidemic in Réunion.[49]

The major vector involved in the outbreak on Réunion was *Aedes albopictus,* whereas *Aedes aegypti* is implicated as a vector in urban epidemics in India and elsewhere in Asia. Thus two lineages of CHIKV coexist: Asian strains adapted best to *A. aegypti* as a vector and Indian Ocean strains adapted to *A. albopictus.* A single amino-acid mutation in the E1 envelope glycoprotein was associated with adaptation of African CHIKV strains to *A. albopictus* mosquitoes.[50] Isolates obtained from India in 2011 contained at least two additional mutations in the E2 glycoprotein that further promote replication in *A. albopictus.*[51] Because *A. albopictus* is better adapted to temperate climates than is the *A. aegypti* species, the potential for epidemic spread to nontropical regions of the world was enhanced by these adaptive mutations. CHIK fever was imported to northern Italy by a traveler, and 205 cases occurred by autochthonous spread in that region between July and September 2007.[52] Similar data up to 2012 suggest limited spread in southern France. Most countries in Europe have by now documented cases of CHIK fever in travelers returning from epidemic regions.

Between 1995 and 2005, only four cases of CHIK fever among travelers returning to the United States were reported to the CDC. In contrast, 106 cases were identified from 2006 to 2009.[53] Sixty-two of these subjects had recently visited India, and 13 had CHIKV viremia on arrival in the United States; 12 of these were returning to states that reported the presence of potential CHIKV vector mosquitoes. No endogenous spread of CHIK fever had been reported in the United States or its territories up to 2012. However, in October 2013, Asian-linked CHIK virus was introduced into St. Martin, spread to Martinique and Guadeloupe, and affected nearly every island in the Caribbean, including Puerto Rico. In 2014, a peak of 4710 cases of CHIK fever were reported to the CDC from US territories and 2811 cases from the US mainland, with 12 endogenous cases in Florida. This seems to have been the peak of the outbreak in the United States and territories. Since then, case reports to the CDC have waned in number on a year-to-year basis; in 2017 only 104 cases, all thought to have been imported, were reported from 24 states, with only 33 cases reported in US territories.[46]

Asian lineage CHIKV also appeared in multiple Central and South American countries in 2013, probably imported from Micronesia.[46,54] As of April 2016, the Pan American Health Organization reported that local transmission of CHIK had occurred in 45 countries or territories throughout the Americas, with more than 1.7 million suspected cases in the affected countries, including Colombia, Venezuela, and Brazil, and with cases reported from nearly all other countries of the northern half of South America as well, most recently in Argentina.[54,55] Most of these countries were also experiencing simultaneous outbreaks of dengue virus, which is endemic to the area, and Zika virus. All three viruses are vectored by the same *Aedes* mosquitoes and cause very similar, nearly clinically indistinguishable, disease states in their simplest presentation.

Serologic survey results suggest that epidemics occur periodically when the youngest group of inhabitants of an endemic area is susceptible. The following factors suggest the potential for future major CHIK fever epidemics, especially in the Western hemisphere: (1) the increasing prevalence of Aedes species, especially *A. albopictus,* in North and South America and Europe[47]; (2) the increasing mobility of populations residing in endemic or currently epidemic areas; (3) the lack of a requirement for an animal host, other than humans, to support the CHIKV life cycle; and (4) the presence of large populations of immunologically naïve individuals in the United States. However, it can also be said that the threat of autochthonous spread of CHIK fever on the US mainland seems to have abated as of 2017, despite the apparently favorable conditions for outbreaks in eastern regions of the United States harboring *Aedes* mosquitoes.

O'nyong-Nyong Virus

O'nyong-nyong virus is closely related to CHIKV, both antigenically and genetically. It initially appeared in Uganda in the form of an epidemic that involved 2 million people in its final extent by the middle-to-late 1960s. The virus then disappeared until 1996, when it reappeared in the context of a second epidemic in southern Uganda. The reemerging virus was shown by nucleotide sequence analysis to be closely related to the 1959 strain.[56] During the fall of 2003, there was a third outbreak of O'nyong-nyong fever in the Ivory Coast that was initially mistaken for measles.[57] A 2010 serologic survey conducted in Cameroon demonstrated that O'nyong-nyong virus continues to circulate at a low level in the absence of a frank epidemic, suggesting that most infections are asymptomatic or only mildly so.[58] O'nyong-nyong virus and CHIKV are closely related to a third virus, Igbo-Ora, also found in Africa. Risk factors for O'nyong-nyong fever include residence in rural villages where the vector *Anopheles gambiae* mosquitoes congregate. A nonhuman primate reservoir of infection has not been identified.

Sindbis Virus

SINV was first isolated in Egypt in 1952. It is transmitted among birds by *Culex* and *Culiseta* mosquitoes. Studies in South Africa show that extensive human disease occurs in parallel with years of abundant rainfall in association with flooding of usually arid regions. Thus infected mosquitoes and susceptible humans are presumably brought into proximity. Infection rates may approach 15% during a major transmission season. SINV and the flavivirus West Nile virus share the avian–*Culex* mosquito hosts. In South Africa, the Nile Valley of Egypt, and Israel, individuals with antibodies to SINV frequently also have antibodies to West Nile virus.

Besides the indicated countries of Africa, SINV also causes a fever/arthralgia/rash syndrome in northern Europe, China, and Australia, as well as in the Philippines. In northern Europe, symptomatic SINV infection is recognized in the region between 60° and 65° north latitude in Sweden (as Ockelbo disease), Finland (as Pogosta disease), and the Commonwealth of Independent States (as Karelian fever). These are a single disease entity characterized by arthritis, pruritic rash, fatigue, mild fever, headache, and muscle pain. The causative agent is a northern European strain of SINV, genetically related to SINVs isolated in Africa.[59] Older adults who work or vacation in forested areas are most often affected. The virus has been isolated from *Culiseta, Aedes,* and *Culex* mosquitoes. The grouse is the major avian host of SINV in Finland.[60] Outbreaks of Pogosta disease occurred every 7 years, between 1974 and 2002, for reasons that may have to do with the migratory habits of grouse between South Africa and northern Europe. For example, 1301 and 597 cases were documented in 1995 and 2002, respectively. However, the expected outbreak in 2009 resulted in many fewer cases (105) than were expected based on the outbreaks recorded in 1995 and 2002.[61] Debilitating arthritis following acute Pogosta disease may persist for years. It is positively associated with the human leukocyte antigen subtype DBR1*01 and the presence of autoantibodies.[62]

Ross River Virus and Barmah Forest Virus

RRV is a cause of epidemic polyarthritis, myalgia, and fatigue in humid northern tropical areas of Australia and neighboring Pacific Islands. It is the most common and most widespread arboviral disease in Australia. Joint symptoms are especially intense and may last as long as 3 years after fever and rash have abated. RRV is transmitted by a wide variety of mosquito species; the life cycle principally requires mosquito vectors and marsupial hosts, especially the kangaroo and the wallaby, but dogs, cats, and possums are also implicated.[63] Human disease is seasonal in occurrence. A second alphavirus, BFV, is also found in northern Australia and shares mosquito vectors and animal hosts with RRV. BFV causes, in general, a less severe arthritis that is shorter in duration than that caused by RRV.[64] Both diseases were increasing in incidence as of 2013. RRV and BFV were implicated in 6546 disease reports between July 2009 and June 2010, probably due to flooding that enhanced vector mosquito replication.[65] In 2015, Queensland reported the worst outbreak of RRV in 20 years, with 2835 cases recorded in the first 3 months of 2015, mostly among young adults; more than 1000 cases had been reported in Victoria during the first 2 months of 2017.[66]

Mayaro Virus

Mayaro virus was first isolated in the Caribbean in the 1950s in association with an epidemic of febrile illness with rash and occasional arthropathy.

It has since caused epidemics in Brazil and Bolivia. The virus has been isolated from *Haemagogus* mosquitoes and from marmosets, as well as other nonhuman primates.[67] These may provide a reservoir for virus in the natural setting. As for other human pathogenic flavi- and alphaviruses, there is cause for concern regarding epidemic spread; a serosurvey in rural areas of Brazil recently identified 55% of febrile patients (15 of 27), in an outbreak thought to have been caused by CHIKV or dengue virus, as positive for Mayaro virus–specific immunoglobulin M (IgM), suggesting a cause-effect relationship.[68]

A long list of alphaviruses, including Bebaru, Cabassou, Getah, Kyzylagach, Middelburg, Nduma, Pixuna, Sagiyama, Semliki Forest, Una, and Whataroa viruses, either are not known to cause human disease or cause disease of the fever/arthropathy type that is rare.

PATHOGENESIS

Encephalitis

The locus of alphavirus replication in the mosquito is the midgut epithelium, which is targeted after the mosquito has taken a blood meal from a viremic host. In the mosquito, the infection is generally thought to be a lifelong, persistent, productive one, although there may be associated necrotic changes in the midgut.[69] In humans, the bite of an infected mosquito results in the deposition of virus in cutaneous tissues. Muscle cells and Langerhans cells are sites of replication. Infected Langerhans cells deliver virus to lymph nodes and ultimately to the blood.[1] The initial phase of infection is marked by viremia and a febrile response, signaling the replication of virus in nonneural tissues. Alphaviruses stimulate the innate immune response via interaction with Toll-like receptors and because the double-stranded RNA generated in the course of alphavirus RNA replication is a potent IFN inducer. The innate response and the capacity of the virus to suppress or evade it are major determinants of outcome for all alphavirus infections.

Although it does not commonly invade the human CNS, mouse brain–adapted SINV causes encephalitis in mice that has been intensively studied as a model for alphavirus encephalitides in humans. The degree of neurovirulence is different for different inbred strains and for different SINV strains, and is inversely related to age; disease is fatal in neonates.[70] The initial event in a fatal encephalomyelitis appears to be infection of capillary endothelial cells, after which virus quickly spreads throughout the CNS, especially involving neurons of the olfactory tract and hippocampus and motor neurons of the brainstem and spinal cord. Peak titers of virus are seen 2 to 3 days after infection in the brain and spinal cord. In neonates, neuronal cell death is by both the apoptotic and necrotic pathways. Apoptosis is triggered by the fusion event that occurs during virus entry, which induces sphingomyelinases that promote release of proapoptotic ceramide.[71] Mature neurons are more resistant to apoptosis than immature neurons, which may account for the increased susceptibility of very young mice to a fatal outcome.[72] Infected neurons also produce other proapoptotic products (e.g., BCL-2–associated X protein [BAX]), as well as antagonists of apoptosis (e.g., BCL-2, Beclin-1, certain protease inhibitors).[72] Locally produced type III and type I IFNs are important for control of virus replication early in infection, especially IFN-γ (type III) and IFN-β (type I). Conversely, differences in neurovirulence in mice of a highly neurovirulent and a nonneurovirulent strain of SINV correlated with the capacity of nsp1 to suppress the cellular response to IFNs, by disrupting Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling.[73] Mice deficient in IFN-β produce 10-fold higher titers of virus in brain, compared with normal mice. The virus-produced inflammatory response also stimulates interleukin (IL)-10 production from Th17 cells, which seems to protect from immune-mediated cell death without affecting virus replication. In mice infected by a nonlethal strain of SINV, absence of IL-10 led to a shift to Th1 type response, a reduction in the beneficial inflammatory response, and increased morbidity.[74] Other factors produced as part of the innate response include tumor necrosis factor (TNF); IL-1 and IL-6; chemokine (C-C motif) ligand 2 (CCL2), ligand 3 (CCL3), and ligand 5 (CCL5); and chemokine (C-X-C motif) ligand 9 (CXCL9) and ligand 10 (CXCL10).[75] These induce glial cell activation, expression of adhesion molecules on endothelial cells, and cell migration.[76]

Clearance of virus is initiated 4 to 5 days postinfection in nonlethal infection of mice. The adaptive immune response plays the major role in this process and is initiated in peripheral draining lymph nodes. Virus-specific $CD4^+$ and $CD8^+$ T cells rapidly increase, acquire effector functions, and enter the circulation. There is a two-phase B-cell response: a rapid extrafollicular response resulting in low-affinity IgM class antiviral antibody and a slower one emanating from the germinal center of affected lymph nodes, which eventually yields higher-affinity immunoglobulin G (IgG). Virus-specific cytotoxic $CD8^+$ T cells enter the brain within 3 to 4 days after infection and produce IFN-γ, followed by cytokine-producing $CD4^+$ T cells and antibody-secreting $CD19^+$ B cells.[77,78] Cytotoxic T cells do not clear virus-infected neurons in the brain, probably because neurons do not display human leukocyte antigens.[79] However, endothelial cells and ependymal cells in the brain are subject to T-cell surveillance, and cytotoxic T cells do play a major role in clearance of virus-infected cells from motor neurons of the spinal cord. IFN-γ both increases proinflammatory cytokines, leading to clinical disease in the early phase, and synergistically clears virus in the presence of SINV antibodies, partly by increasing chemokine production important for the infiltration of antibody-secreting B cells into the CNS, in late phases of disease.[80]

SINV E2-specific antibodies produced primarily by $CD19^+$ B cells entering the brain are primarily responsible for clearance of virus from neurons of the olfactory tract and hippocampus. After extracellular infectious virus has been eliminated in the brain, E2 antibodies (and IFN-γ) also suppress virus production from infected neurons by preventing the budding of nascent virions and shutting down viral RNA synthesis, although virus RNA persists in cells. Antibody and IFN-γ, operating via the JAK/STAT pathway rather than cytolysis, also restore host cell protein synthesis, membrane integrity, and the capacity to respond to IFN-α/β.[81] In the first several days after infection, the percentage of antibody-secreting B cells specific for SIN antigens increases in the brain, and these specialized B cells are maintained apparently for the life of the animal. Likewise, SINV RNA is detectable indefinitely in neurons of infected mice (>60 days), even though recovered from clinical manifestations of infection. In mice lacking an adaptive response, infectious virus is not cleared and the IFN-α and IFN-β responses are sustained.[77] Various chemical inhibitors of glutamate excitotoxicity protect mice from SINV encephalomyelitis paradoxically by reducing the entry of inflammatory cells into the CNS and delaying virus clearance.[80]

The pathogenesis of encephalitis caused by the New World viruses, EEEV, WEEV, and VEEV, is not nearly as well studied as is the SINV model, possibly because of the level of biosafety that must be observed when working with these viruses in the laboratory. However, available data do suggest there are parallels to the SINV system. For example, mortality due to VEEV is enhanced in mice lacking IFN regulatory factors (IRF-1 and IRF-2), IFN receptors, or type I IFN itself.[82,83] Moreover, VEEV can interdict the capacity of cells to respond to IFN by interrupting JAK/STAT signaling, as for SINV. The earliest adaptive immune response to VEEV is nonneutralizing antibody that mediates clearance of virus-infected cells in the periphery. This is followed by a neutralizing antibody response with E2 specificity. Before CNS invasion in experimental animals, VEEV replicates in lymphoid tissues, especially including dendritic cell types, resulting in necrotic changes, and in bone marrow, resulting in lymphopenia. Lymphoid infection in mice is followed by high-titer viremia, during which the peripheral CNS is seeded, mainly through the olfactory system.[84] VEEV also replicates in the pancreas and salivary glands of experimental animals but does not have a diabetogenic effect in humans who have survived encephalitis. Pathologic changes associated with encephalitis include neuronal cell loss due to apoptosis, mild-to-moderate neutrophilic infiltrate, gliosis, and perivascular cuffing with involvement of Purkinje cells.[85] EEEV causes lesions throughout the brain and spinal cord, most severely involving the cerebral cortex and basal ganglia. WEEV causes focal necrosis in the striatum, globus pallidus, cerebral cortex, thalamus, pons, and meninges. Transplacental spread of VEEV and WEEV may affect the fetus, resulting in massive cerebral necrosis.[86] Although persisting neurologic deficits may be among the sequelae of human infection with VEEV, WEEV, and EEEV, long-term persistence of either infectious virus or virus RNA in the CNS has not been documented.

Fever, Arthralgia, and Rash

During self-limited infection with a nonneurotropic alphavirus, such as CHIKV, viremia may precede the onset of symptoms by several days.

Illness is typically heralded by fever roughly coinciding with the peak of viremia, and the level of viremia closely parallels the fever curve at early times. The fever curve may be "saddleback"; fever may abate 4 to 8 days after onset in concert with the level of viremia and then recrudesce to peak readings for up to 2 weeks. IgM antibody titers rise during the acute phase and remain at a high plateau. As viremia fades and the first phase of the fever subsides, HI and neutralizing IgG antibody titers start to increase, suggesting that these immune responses curtail the acute phase of infection. However, the late and chronic sequelae of CHIKV and RRV occur in the presence of the vigorous humoral response, and pathology is consistent with an autoimmune etiology. (Likewise, symptoms and signs of encephalitis occur in the late phases of infection by EEEV and VEEV.) Biopsy of the maculopapular-to-macular rash in CHIK infection shows lymphocytic perivascular cuffing and extravasation of erythrocytes from superficial capillaries.[87] Arthralgia may accompany early symptoms of fever and malaise (especially in CHIK fever) or may be slightly delayed and quite severe, leading to frank arthritis (for both CHIK fever and RRV disease). Virus RNA and viral antigens can be detected in joint tissues as well as inflammatory cell infiltrates, consisting primarily of monocytes and virus-infected macrophages within synovial effusions.[88,89] Similarly, CHIKV antigens have been detected in muscle biopsy specimens exhibiting severe myositis with inflammatory macrophages, T lymphocytes, and myofibril pathology.[90] A general scheme for the time course of the pathogenesis of acute alphavirus diseases is shown in Fig. 151.2.

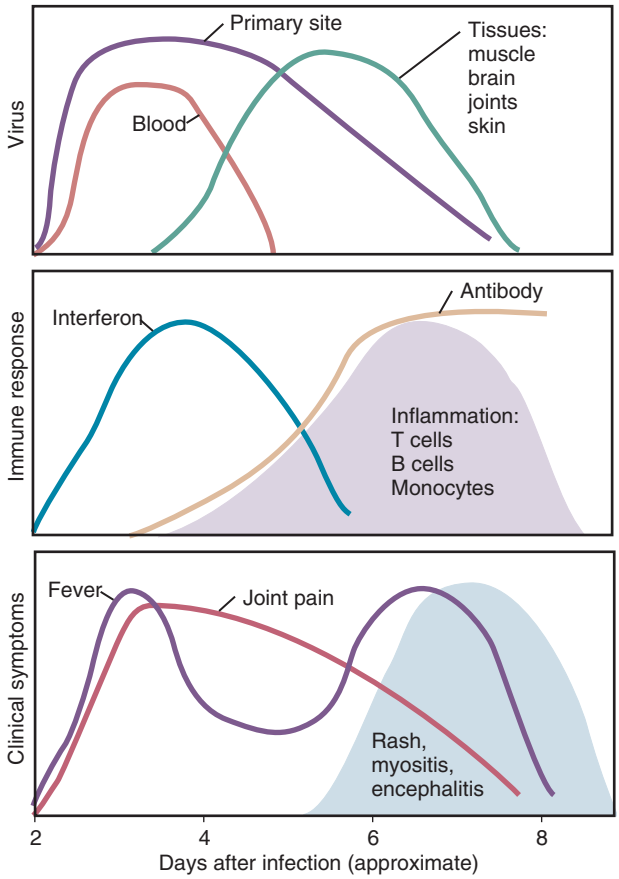

FIG. 151.2 Schematic diagram of the pathogenesis of alphavirus-induced disease. Viremia may be accompanied by production of interferon, other proinflammatory cytokines, and fever. Virus then spreads through the blood to other target tissues. As the immune response is induced, the viremia is terminated, but fever is renewed with the appearance of a mononuclear inflammatory response in the infected tissues. In infections that lead to rash and arthritis, joint pain usually appears early after infection and before the appearance of the rash. *(From Griffin D. The alphaviruses. In: Knipe DM, Howley PM, eds.* Fields Virology. *5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007:1031.)*

Using an established mouse model of RRV disease in humans, macrophage-derived factors were shown to be critical in the development of striated muscle and joint tissue damage. Histologic analyses of muscle and ankle joint tissues demonstrated a substantial reduction in inflammatory infiltrates in infected mice depleted of macrophages. Levels of the proinflammatory factors TNF-α, IFN-γ, and macrophage chemoattractant protein-1 were also dramatically reduced in tissue samples obtained from infected macrophage-depleted mice, compared with samples obtained from infected mice without macrophage depletion. These factors were also detected in the synovial fluid of patients with RRV-induced polyarthritis. Neutralization of these factors reduced the severity of disease in mice, whereas blocking nuclear factor kappa B by treatment with sulfasalazine ameliorated RRV inflammatory disease and tissue damage.[91] The balance between the innate response and the capacity of the virus to blunt that response as a major determinant of outcome seems to hold for RRV, as noted for other alphaviruses. Myeloid differentiation primary response gene 88 *(Myd88)*–dependent Toll-like receptor 7 signaling has a protective effect in RRV-infected mice. Higher viral loads, enhanced tissue damage, and reduced antibody affinity were seen in knockout mice.[92]

The outbreak of CHIK fever in East Africa and its spread into Asia and the New World has renewed interest in CHIK fever pathogenesis. Acute disease correlated with a dramatic increase in IFN-γ and IL-12, and chronic arthritis correlated with prolonged elevated levels of IL-12 in Réunion patients. IL-12 returned to normal in association with recovery.[93] A strong innate and adaptive immune response was associated with recovery among patients in Gabon as well, with elevated levels of IL-4, IL-10, and IFN-γ. A $CD8^+$ T-cell response preceded $CD4^+$ T-cell increases.[94] In vitro studies showed that CHIK (and SIN) nsp2 mediates the ubiquitin-dependent degradation of Rpb1, a component of RNA polymerase II, thus globally downregulating host gene expression, independent of autophagy, apoptosis, and inhibition of STAT1.[95] CHIK nsp2 also inhibits JAK/STAT phosphorylation, blocking IFN-I and IFN-II responses.[96] This is a common ancillary function for nsp2 of the Old World alphaviruses, including SINV and Semliki Forest virus. Other new information from in vitro studies suggests that CHIKV triggers autophagy to promote its replication; that CHIKV evades the host unfolded protein response by suppressing phosphorylation of the translation inhibitory factor eIF2α; and that CHIK nsp3 suppresses stress granule assembly in infected cells.

A mouse model for CHIK fever–related arthritis has been described in which the mice display evidence of arthritis, tenosynovitis, myositis, and virus persistence in the ankle joint distal to the virus-inoculated footpad.[97] In the mouse model, the dendritic cell immunoreceptor (DCIR) affects CHIKV pathogenesis. DCIR is a negative regulator of the host inflammatory response expressed on dendritic cells, monocytes, macrophages, B cells, and neutrophils.[98] The capacity of dendritic cells to express DCIR downregulated the inflammatory response and affected cytokine expression; IL-10 and IL-6 expression was increased, IL-12 expression was reduced, and virus replication was enhanced at late times in mice lacking DCIR. In a complementary study, $CD4^+$ T cells were seen to mediate joint inflammation in mice, independent of IFN-γ secretion.[99] In addition, IRF-3 and IRF-7 were shown to protect mice against CHIKV hemorrhagic fever and shock. IRF-3 and -7 knockout *($IRF3/7^{-/-}$)* mice developed high virus titers, edema, vasculitis, hemorrhage, fever and hypothermia, oliguria, and thrombocytopenia, indicating once again the importance to the host of the innate response as defense against pathogenic alphaviruses.[100] Recombinant activating gene 1 knockout *($RAG1^{-/-}$)* mice were recently shown to exhibit a chronic CHIKV infection and used to evaluate a candidate live-attenuated virus CHIK vaccine.[101]

CLINICAL MANIFESTATIONS

Alphaviruses Causing Encephalitis

EEE is heralded by a 5- to 10-day prodrome of headache, high fever, chills, nausea, and vomiting or diarrhea. In patients in whom CNS involvement develops, initial symptoms are followed by mental confusion and somnolence often accompanied by photophobia. Seizures or convulsions occur most often in younger patients. Seizures are usually tonic-clonic, but may also be of the complex partial type. Progression to frank

coma can occur rapidly. Physical examination may reveal nuchal rigidity, depressed or hyperactive reflexes, tremors, muscle twitching, spastic paralysis, bilateral papilledema, and cranial nerve palsies, which can be secondary to increased cerebrospinal fluid (CSF) pressure or directly related to inflammation. Cranial nerves VI, VII, and XII are most often affected. Infants may develop bulging fontanelles. Other occasional findings include cyanosis secondary to depressed respiratory drive and facial, periorbital, or generalized edema.[35,102]

Laboratory findings in acute EEE are as follows[35,102]: a polymorphonuclear leukocytosis is present in most cases to levels of 15,000 cells/mm^3 or higher. Hyponatremia, when noted, may be due to the syndrome of inappropriate antidiuretic hormone. CSF protein is increased, and 500 to 2000 cells/mm^3 are present, mostly lymphocytes. Red blood cells are occasionally noted as well. Hypoglycorrhachia is not present. Serologic tests are positive for antibodies to EEEV (see "Diagnosis" later).

Neurologic sequelae include mental retardation, behavioral changes, convulsive disorders, and paralysis. These may occur in 70% of patients recovering from EEE infection. Negative prognostic signs in EEE are age older than 40 years, rapid progression to coma, severe hyponatremia, and CSF cell counts higher than 500/mm.[35,102] Among patients who recover, sequelae are less frequent in adults.

WEE infection is also heralded by a short prodromal phase lasting 1 to 4 days. Signs and symptoms during this period are similar to those described for EEE. In adults, the prodromal phase may subside spontaneously with no neurologic complications. In subjects who do progress, the course of encephalitis resembles that of EEE as well, except that focal neurologic abnormalities may be less common.[35,102] Laboratory studies usually reveal a polymorphonuclear leukocytosis, but counts are significantly lower than those noted for EEE. The CSF protein is usually increased, and lymphocytes and red blood cells are present. Glucose is normal. Neurologic sequelae occur in 30% of young patients and are similar to those noted for EEE.[103] Parkinsonism is an occasional late sequela of WEE in adults.

The most common clinical manifestation of epizootic VEE infection is a febrile illness with malaise after an incubation period of 1 to 6 days. Chills, myalgia, and headache with or without photophobia, hyperesthesia, and vomiting are common. Occasionally patients report a sore throat. Fever may remit in a short time, with recrudescence the next day. Approximately 4% of children and less than 1% of adults progress to severe encephalitis, which usually occurs after a few days to a week of the prodromal illness.[37,38] Features of encephalitis include nuchal rigidity, ataxia, convulsions, coma, and paralysis, in ascending order of severity. Laboratory studies characteristically reveal lymphopenia, sometimes accompanied by neutropenia and mild thrombocytopenia within a day or two of onset. Serum glutamic-oxaloacetic transaminase (aspartate aminotransferase) and lactate dehydrogenase enzymes are typically increased. CSF examination reveals a few hundred lymphocytes. The overall case-fatality rate is less than 1% but approaches 20% in those who progress to encephalitis. Nearly all individuals in endemic areas contract enzootic VEE infection, as suggested by the results of serologic surveys.[38] Most seem to have experienced the influenza-like prodromal illness or to have had asymptomatic infection.

Alphaviruses Causing Fever, Polyarthritis, and Rash

CHIK fever is taken as the prototype of the diseases caused by this large group of alphaviruses. This is an acute viral infection characterized by a rapid transition from a state of good health to illness that includes severe arthralgia and fever.[104,105] The incubation period ranges from 1 to 12 days. Body temperature increases abruptly to as high as 40°C and is often accompanied by shaking chills. After a few days, fever may abate and recrudesce, giving rise to a "saddleback" fever curve (see Fig. 151.2). Arthralgia is polyarticular, favoring the small joints and sites of previous injuries, and is most intense on arising. Patients typically avoid movement as much as possible. Joints may swell without significant fluid accumulation. These symptoms may last from 1 week to several months and are accompanied by myalgia. The rash characteristically appears on the first day of illness, but onset may be delayed. It usually arises as a flush over the face and neck, which evolves to a maculopapular or macular form that may be pruritic. The latter lesions appear on the trunk, limbs, face, palms, and soles, in that order of frequency. Petechial skin lesions have also been noted. Headache, photophobia, retro-orbital pain, sore throat with objective signs of pharyngitis, nausea, and vomiting also occur in this setting. Laboratory test results may reveal a mild leukopenia with relative lymphocytosis. The erythrocyte sedimentation rate is usually markedly elevated, and the C-reactive protein is positive.[105,106] Severe arthritic involvement is most commonly seen in adults, whereas children occasionally present with symptoms referable to the CNS, including seizures and convulsions. Long-term joint involvement has been reported in association with human leukocyte antigen B27.[106]

As a result of the recent CHIKV pandemic, additional clinical aspects of the disease have become evident. In 88 patients on Réunion Island who were assessed a median of 18 months after onset of disease, 56 reported persistent arthralgia and half of those reported a resulting reduction in their ability to carry out daily activities. All had polyarticular involvement, and pain was continuous in more than half of those who reported arthralgia. Approximately 40% of the total had persisting CHIK-specific IgM antibodies. Transient ocular involvement was described among adult patients in South India, with the main manifestations including anterior uveitis, optic neuritis, retrobulbar neuritis, and dendritic lesions.[107] In another study emanating from Réunion Island, atypical cases of CHIKV infection were defined as those in which patients developed symptoms other than fever and arthralgia. A total of 610 such cases were studied, among which 222 were graded as severe. Of these, 65 patients died. Atypical manifestations included meningoencephalitis, hepatitis, bullous dermatitis, pneumonia, and diabetes.[108] According to a second report from Réunion, serious acute CHIKV infection resulted in encephalitis (14 cases), myocarditis, hepatitis, and Guillain-Barré syndrome among 33 patients who were admitted to an intensive care unit. There was a 48% fatality rate in this severely ill group.[109]

Mother-to-child transmission of CHIKV infection was demonstrated in a retrospective study of neonates. Clinical signs seen in 38 infants included fever (79%), rash (82%), pain (100%), and peripheral edema (58%). Laboratory abnormalities included thrombocytopenia, lymphopenia, decreased prothrombin, and elevation of alanine aminotransferase. Virus was detected in the CSF of 22 of 24 infants evaluated by polymerase chain reaction (PCR); 14 of these subjects had abnormal findings on brain magnetic resonance imaging with white matter lesions or intraparenchymal hemorrhages. Myocardial hypertrophy, ventricular dysfunction, pericarditis, and coronary artery dilatation were documented in a minority of this group, and one neonate died of necrotizing enterocolitis.[110] Among all patients, the case-fatality rate on Réunion was estimated at 1:1000. Higher rates were calculated for Mauritius and parts of India. Also on Réunion, a cohort of 180 patients with proven CHIK fever was followed for 36 months. Sixty percent of patients reported arthralgia or arthropathy with swelling, usually with episodic relapse and recovery, typically affecting several joints in a symmetrical distribution. In 77% of those reporting arthralgia, the symptoms were characterized as highly incapacitating. Age older than 35 years and presence of arthralgia at 4 months after the acute illness were risk factors for long-term arthropathy. These patients did not display biologic markers commonly associated with autoimmune disease/rheumatoid arthritis, in contrast to results reported earlier from a study of chronic persisting CHIK fever–related arthritis of 18 months' duration.[106,111]

Cases of CHIK fever among travelers returning to their home countries from the epidemic areas have been characterized. In one study from Germany, CHIKV infection was documented in 20 of 69 patients who initially reported CHIK-like symptoms, including fever and arthralgia. Two-thirds of the patients had persistent arthralgia for longer than 2 months, and 13% had it for longer than 6 months. Active viremia was reported in all patients who reported to the clinic within the first week of illness.[112] The recent experience with travelers returning from Africa, Réunion, or Asia to Italy or France was mentioned earlier.

DIAGNOSIS

The epidemiology of each of the disease entities caused by alphaviruses is highly specific and provides a major clue to diagnosis. Thus knowledge of the recent travel or outdoor exposure history of the patient is of vital importance. In certain locales, during epidemic spread of a disease, the diagnosis is obvious. In the United States, the initial signs and symptoms

of EEE or WEE infection may mimic those of enteroviruses. Encephalitis caused by the flaviviruses West Nile virus and St. Louis encephalitis virus may occur in the same setting as EEEV encephalitis. CDC criteria for the diagnosis of an arboviral encephalitis require the presence of an acute febrile illness with encephalitis during a time when virus transmission is likely, plus one of the following criteria: (1) greater than fourfold increase in viral antibody titer between acute and convalescent sera; (2) virus isolation from CSF, blood, or tissue; or (3) IgM positive to the virus in CSF.

EEEV can sometimes be isolated from serum during the prodrome,[35] but historically most cases are diagnosed by testing paired sera in HI tests or in a neutralization assay because only low-level viremia occurs in human subjects. Convalescing patients may manifest high complement fixation antibody titers, and IgM antibodies can be detected by enzyme-linked immunosorbent assay (ELISA).[113] Magnetic resonance imaging with specialized imaging techniques such as fluid-attenuated inversion recovery and T2 weighting is of value for both diagnosis and following the clinical course of encephalitis.[114] EEE caused focal radiographic changes involving the basal ganglia, thalamus, and brainstem, in descending order of frequency, in one study of 36 cases.[102]

Diagnostic testing for WEE follows a similar pattern except that viremia is usually not detectable. The presence of WEE in a specimen may be documented by inoculating suckling mice or embryonated eggs. In contrast, sera taken from patients with VEE infection within 48 hours of onset are usually positive for virus. However, sera from patients with full-blown encephalitis are usually negative, and the diagnosis may be made by complement fixation testing. ELISA for VEE-specific IgM in sera and CSF is available. IgM and IgG ELISAs using attenuated VEE as antigen are the most sensitive diagnostic tests but should be followed up with the plaque reduction neutralization assay to prove specificity.[115]

Sensitive nucleic acid amplification assays using the reverse-transcriptase PCR (RT-PCR) exist for rapid diagnosis of EEE, WEE, VEE, and West Nile virus infections.[116] Most recently, ELISA and RT-PCR have been combined in a single, rapid-detection system that uses biotinylated oligonucleotide probes complementary to unique amplified nucleotide sequences in the WEE, EEE, VEE, and Mayaro virus genomes.[117] Human monoclonal antibodies directed against epizootic VEEVs have been expressed in bacteriophage and are being used for rapid diagnostic assays.[118] When these assays are available, they constitute a rapid substitute for actual culturing of virus from a clinical specimen.

CHIK fever in its acute presentation or in mild disease may be clinically indistinguishable from disease caused by the alphaviruses (Mayaro virus, O'nyong-nyong virus, RRV, or SINV) or by the flaviviruses (dengue virus and Zika virus). Dengue virus and CHIKV can coinfect mosquitoes, and simultaneous transmission of both viruses to humans has been documented. In one clinical study conducted in Singapore, where dengue virus and CHIK now cocirculate,[119] CHIK fever was distinguished from dengue fever (DF) or dengue hemorrhagic fever (DHF) on the basis of clinical and laboratory criteria. Bleeding, sore throat, cough, nausea, vomiting, diarrhea, abdominal pain, anorexia, and tachycardia were more common in DF or DHF than in CHIK fever, whereas joint pain was more common in CHIK fever. Laboratory results showed that hemoconcentration, thrombocytopenia, lymphopenia, and liver enzyme elevations were also more common in DF or DHF than in CHIK fever. In addition to DF and DHF, parvovirus B19 infection, the prodrome of hepatitis B, juvenile rheumatoid arthritis, and rubella, may also be confused with CHIK fever or with the other alphavirus infections. Patients with CHIK fever are usually viremic for the first 48 hours, and the virus is easily isolated by in vivo or in vitro methods. Viremia may be so intense (titer $>10^7$ plaque-forming units/mL of blood) as to yield measurable amounts of hemagglutinating activity from sera.[120] Consequently, virus in sera can also often be detected by ELISA directly.[121] As previously mentioned, the decrease in levels of viremia parallels a rapid increase in titers of HI and neutralizing antibodies. ELISA testing for virus-binding antibodies may detect cross-reactive immune responses to other alphaviruses. The recent CHIK virus epidemics in Africa and Asia have stimulated the development of rapid diagnostic tests on the basis of variations of the real-time RT-PCR, with particular emphasis on their ability to distinguish dengue from CHIK.[121,122] A recent study of the antibody response to CHIK proteins in Singaporean patients revealed high-titer neutralizing activity and responses to E1, E2, E3, and nsp3 in six patients at 2 to 3 months postinfection. Only the response to E2 was still significant at 21 months postinfection, and it seemed to account for persisting capacity of sera to neutralize virus at high dilutions.[123]

THERAPY AND PREVENTION

There is no licensed product approved for treatment of any of the diseases caused by alphaviruses. Low-dose steroids have been used to treat arthritis following RRV infection, with the attendant risks and side effects. Chloroquine has proven ineffective in treatment of CHIK fever–related arthritis, but there has been some modest success with TNF blockers.[124] Harringtonin, a cephalotaxine alkaloid, ameliorates SIN arthritis in mice and inhibits CHIKV replication in vitro; it is under further study.[125] Monoclonal antibodies are currently of major interest as potential prophylactic or therapeutic agents in CHIK fever.[126] Because the clinical and laboratory findings of alphavirus arthritides bear some resemblance to rheumatoid arthritis, there is some hope for crossover effectiveness of drugs used to treat the latter.[124] For encephalitis, supportive measures and intensive nursing care are indicated. Analgesics and nonsteroidal antiinflammatory drugs remain the main treatment options for symptoms of arthritogenic alphavirus diseases.

Prevention of WEE and EEE depends primarily on control of vector mosquito populations. During outbreaks, susceptible individuals engaged in high-risk activities should be advised to avoid exposure as much as is practicable by using effective mosquito repellents and netting, wearing full-length trousers and long-sleeved shirts, and avoiding outdoor activities, at least during periods of maximal mosquito activity. Inactivated vaccines against EEE and WEE (and against VEE and CHIK) were developed at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) several decades ago, for use in laboratory workers and other personnel who might be exposed to infectious aerosols. Although acceptably safe, these vaccines induce a relatively short-lived immune response, as measured by an in vitro assay for virus neutralizing antibodies in sera. Periodic booster doses are necessary to maintain seropositivity.[127] There has been no attempt to license these vaccines for widespread use. An inactivated EEE/WEE veterinary vaccine, administered as a component of a multivalent vaccine, is used against EEE in horses and birds and against WEE in horses.

Both formalin-inactivated and live-attenuated vaccines to prevent VEE are in limited use in humans. The live-attenuated VEE vaccine strain TC-83 is used in the diagnostic ELISA, and large-scale vaccination of horses with this strain is the major approach to the prevention of VEE epizootics. A live-attenuated vaccine to prevent CHIK fever (strain TSI-GSD-218) has been shown to be safe in volunteers at the level of phase II clinical trials.[128] One study in which the experimental live-attenuated VEE and CHIK fever vaccines were sequentially administered to human volunteers indicated that preexisting alphavirus immunity interferes with a subsequent neutralizing antibody response to a heterologous vaccine virus.[129] Similarly, the inactivated EEE and WEE vaccines described above interfere with each other if administered on the same day, and with the live VEE vaccine if given shortly prior to a dose of the latter vaccine.[130]

The live VEE and CHIK vaccine candidates mentioned previously were derived in the early 1990s by serial passage of virulent human isolates in tissue culture, and neither one is licensed by any regulatory authority. Newer approaches to alphavirus vaccine development, stimulated especially by the worldwide spread of CHIKV, have involved the use of recombinant DNA technology. Attenuated mutant VEEVs have been developed with a goal to both prevent VEE and use VEE as a vector for presentation of foreign antigens, because of the propensity of VEEV to target dendritic cells. One viable mutant virus derived from VEE infectious DNA, which contains mutations affecting cleavage of the viral structural proteins, was avirulent in mice, produced solid mucosal immunity against VEE, and currently shows promise primarily as a vaccine for horses.[131] This genetic backbone provided the basis for a number of VEE DNA-based vaccines designed to induce immunity to foreign viral and nonviral antigens, especially when mucosal immune responses are considered to be of crucial importance for protection,[132] but there has been little progress in clinical development of these vaccines in recent years.

Novel vaccines to prevent EEE and several to prevent CHIK fever were recently described, and CHIK vaccine development was recently reviewed.[133] In one approach, the Sindbis structural protein genes were replaced by those of EEEV or CHIKV, in the context of a Sindbis infectious DNA.[134,135] In another, a live-attenuated CHIKV was derived by insertion of the internal ribosome entry site from the encephalomyocarditis virus genome at the 5′ terminus of the CHIK genome to control translation.[136] Modified vaccinia Ankara has recently been used to vector CHIKV antigen.[133] Two groups are developing CHIK vaccines based on virus-like particles.[137,138] Virus-like particles resemble native virus particles by structural and antigenic criteria, but they are nonreplicating and cannot cause disease. One of these two candidate vaccines was shown to protect monkeys from CHIK viremia and is currently in clinical trials.[136] A recombinant measles virus–based CHIK vaccine recently underwent a phase I clinical trial and was found to be immunogenic and to have a generally acceptable tolerability profile.[139] An alphavirus specific for insect cells, Eilat virus, was genetically modified to express the CHIKV surface antigen genes. The resulting chimeric virus can be produced in insect cells and is unable to replicate in vertebrate cells.[140] There is little doubt that one or more of these strategies can result in a safe, effective CHIK vaccine, if development is further pursued.

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The complete reference list is available online at Expert Consult.

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