Chapter 12

ALPHAVIRUS ENCEPHALITIDES

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INTRODUCTION

During the 1930s, three distinct but antigenically related viruses recovered from moribund horses were shown to be previously unrecognized agents of severe equine encephalitis. Western equine encephalitis (WEE) virus was isolated in the San Joaquin Valley in California in 1930[1]; eastern equine encephalitis (EEE) virus was isolated in Virginia and New Jersey in 1933[2,3]; and Venezuelan equine encephalitis (VEE) virus was isolated in the Guajira Peninsula of Venezuela in 1938.[4] By 1938 it was clear that EEE and WEE viruses were also natural causes of encephalitis in humans.[5-7] Naturally acquired human infections with VEE virus occurred in Colombia in 1952 in association with an equine epizootic.[8]

Although these viruses cause similar clinical syndromes in horses, the consequences of the infections they cause in humans differ. EEE is the most severe of the arboviral encephalitides, with case fatality rates of 50% to 70%, and neurological sequelae are common in survivors. WEE virus appears to be less neuroinvasive but has a pathology similar to that of EEE in patients with encephalitis. In contrast, severe encephalitis resulting from VEE virus is rare in humans except for children. In adults, the VEE virus usually causes an acute, febrile, incapacitating disease with prolonged convalescence.

The three viruses are members of the *Alphavirus* genus of the family *Togaviridae*. As with most of the alphaviruses, VEE, EEE, and WEE are transmitted by mosquitoes and maintained in cycles with various vertebrate hosts. Environmental factors that affect the interactions of the relevant mosquito and reservoir host populations control the natural epidemiology of these viruses. Of the 32 viruses classified within this group, VEE, EEE, and WEE are the only viruses regularly associated with encephalitis. Although these encephalitic strains are restricted to the Americas, as a group, alphaviruses have worldwide distribution and include other epidemic human pathogens. Among those pathogens, chikungunya virus (Asia and Africa), Mayaro virus (South America), O'nyong-nyong virus (Africa), Ross River virus (Australia), and Sindbis virus (Africa, Europe, and Asia) can cause an acute febrile syndrome often associated with debilitating polyarthritic symptoms.

Although natural infections with the encephalitic alphaviruses are acquired by mosquito bite, these viruses are also highly infectious by aerosol. VEE virus has caused more laboratory-acquired disease than any other arbovirus. Since its initial isolation, at least 150 symptomatic laboratory infections have been reported, most of which were known or thought to be aerosol infections.[9] Before vaccines were developed, most laboratories working with VEE virus reported disease among their personnel. In one incident reported in 1959 at the Ivanovskii Institute in Moscow, in the former Soviet Union, at least 20 individuals developed disease within 28 to 33 hours after a small number of vials containing lyophilized virus were dropped and broken in a stairwell.[10,11] The ability of aerosolized EEE and WEE viruses to infect humans is less certain, although the possibility is implied from animal studies. Additionally, WEE viruses are less commonly studied in the laboratory than VEE virus, and fewer human exposures may explain the lower incidence of laboratory-acquired infections.

Perhaps as a consequence of their adaptation to dissimilar hosts in nature, the alphaviruses replicate readily and generally to high titers, in a wide range of cell types and culture conditions. Virus titers of 1 billion infectious units per milliliter are not unusual, and the viruses are stable in storage and in a variety of laboratory procedures. Because they can be easily manipulated in the laboratory, these viruses have long served as model systems to study various aspects of viral replication, pathogenesis, induction of immune responses, and virus–vector relationships. As a result, the alphaviruses are well described, and their characteristics are well defined.[12,13]

The designers of offensive biological warfare programs initiated before or during World War II[14] recognized that the collective in-vitro and in-vivo characteristics of alphaviruses, especially the equine encephalomyelitis viruses, lend themselves well to weaponization. Although other encephalitic viruses could be considered as potential weapons (eg, the tick-borne encephalitis viruses), few possess as many of the required characteristics for strategic or tactical weapon development as the alphaviruses:

- These viruses can be produced in large amounts in inexpensive and unsophisticated systems.
- They are relatively stable and highly infectious for humans as aerosols.
- Strains are available that produce either incapacitating or lethal infections.
- The existence of multiple serotypes of VEE and EEE viruses, as well as the inherent difficulties of inducing efficient mucosal immunity, confound defensive vaccine development.

The equine encephalomyelitis viruses remain as highly credible threats, and intentional release as a small-particle aerosol, from a single airplane, could

be expected to infect a high percentage of individuals within an area of at least 10,000 km^2. Furthermore, these viruses are readily amenable to genetic manipulation by modern recombinant DNA technology. This characteristic is being used to develop safer and more effective vaccines,[15,16] yet, in theory, it could also be used to increase the weaponization potential of equine encephalomyelitis viruses.

HISTORY AND SIGNIFICANCE

Descriptions of encephalitis epizootics in horses thought to have been caused by EEE virus were recorded as early as 1831 in Massachusetts.[17] However, it was not until the outbreaks of EEE in Delaware, Maryland, and Virginia in 1933 and 1934 that the virus was isolated. During a similar outbreak in North Carolina in 1935, birds were first suspected as the natural reservoir.[18] The initial isolation of EEE virus from a bird[19] and from *Culiseta melanura* mosquitoes,[20] the two major components of the EEE natural cycle, were both reported in 1951. Outbreaks of EEE virus have occurred in most eastern states and in southeastern Canada but have been concentrated along the eastern and Gulf coasts. Although only 211 EEE cases in humans were reported[21] between 1938 and 1985, the social and economic impact of this disease has been larger than might be expected because of the high fatality rate, equine losses, extreme concern among individuals living in endemic areas during outbreaks, and the surveillance and mosquito-control measures required. Isolation of EEE virus from *Aedes albopictus* mosquitoes, which were recently introduced into EEE endemic areas in the United States, has heightened concern because of the opportunistic feeding behavior of these mosquitoes and their apparent high vector competence for EEE virus.[22]

The initial isolation in 1930 of WEE virus from the brain tissues of a horse with encephalitis was made during a large and apparently unprecedented epizootic in California, which involved at least 6,000 horses with an approximate mortality of 50%.[1] Cases of human encephalitis in California were not linked to WEE until 1938, when the virus was isolated from the brain of a child. During the 1930s and 1940s, several other extensive epizootics occurred in western and north-central states, as well as Saskatchewan and Manitoba in Canada, and affected large numbers of equines and humans. For example, it has been estimated that during 1937 and 1938, more than 300,000 equines were infected in the United States, and in Saskatchewan, 52,500 horse infections resulted in 15,000 deaths.[23,24] Unusually high numbers of human cases were reported in 1941: 1,094 in Canada and 2,242 in the United States. The attack rate in these epidemics ranged from 22.9 to 171.5 per 100,000, with case fatality rates of 8% to 15%.[24]

In the early 1940s, workers isolated WEE virus from *Culex tarsalis* mosquitoes[25] and demonstrated the presence of specific antibody to WEE virus in birds,[26] suggesting that birds are the reservoirs of the virus in nature. The annual incidence of disease in both equines and humans continues to vary widely, which is indicative of an arthropod-borne disease. Significant epidemics occurred in 1952, 1958, 1965, and 1975.[24]

VEE virus was initially isolated during investigations of an epizootic occurring in horses in Venezuela in 1936, and the isolate was shown to be antigenically different from the EEE and WEE viruses isolated previously in the United States.[4,27] Over the following 30 years, many VEE outbreaks were reported among horses, and humans became infected in large numbers in association with these epizootics.[28] Most of those infected recovered after suffering an acute, febrile episode, but severe disease with encephalitis and death also occurred, mostly in children and older individuals. Major epizootics occurred in Venezuela, Colombia, Peru, and Ecuador in the 1960s, apparently spreading to Central America in 1969.[29] These epizootics and previous ones were associated with costly and dire consequences, especially among rural people, who not only had the disease but also lost their equines, which were essential for transportation and agriculture. Between 1969 and 1971, epizootics were reported in essentially all of Central America and subsequently continued north to Mexico and into Texas. The most recent major epizootic occurred in Venezuela and Colombia in 1995.[30]

Between active epizootics, it was not possible to isolate the equine virulent viruses. During the 1950s and 1960s, however, several other attenuated, antigenically different VEE strains were isolated from different geographical areas. These enzootic strains could be differentiated antigenically not only among themselves but also from the epizootic strains.[31] Enzootic strains used different mosquito vectors than the epizootic strains[32] and used rodents as reservoir hosts.[33] Many of the enzootic strains, however, proved equally pathogenic for humans.

Therefore, within 30 years of the initial isolation of the EEE, WEE, and VEE viruses, an accurate picture had emerged of their endemic and epidemic behavior, arthropod vectors, reservoir hosts, and the diseases produced. Although not yet understood at the molecular level, these three viruses were well described as agents of disease, and the basic methods

for their manipulation and production were known. The development of this knowledge occurred during the same period of war and political instability that fostered the establishment of biological warfare programs in the United States[34] and elsewhere, and it was evident that the equine encephalomyelitis viruses were preeminent candidates for weaponization. The viruses were incorporated into these programs for both potential offensive and defensive reasons. The offensive biological warfare program in the United States was disestablished in 1969, and all stockpiles were destroyed[14] by executive order, which stated:

> *The United States shall renounce the use of lethal biological agents and weapons and all other methods of biological warfare. The United States shall confine its biological research to defensive measures such as immunization and safety measures.*[35]

Continuing efforts within the defensive program in the 1960s and 1970s produced four vaccines for the encephalomyelitis viruses: live attenuated (TC-83) and formalin-inactivated (C84) vaccines for VEE, and formalin-inactivated vaccines for EEE and WEE. These vaccines are used under investigational new drug status for at-risk individuals, distributed under investigational new drug provisions, and recommended for use by any laboratory working with these viruses.[9] Although these vaccines are useful, they have certain disadvantages (discussed later in this chapter), and second-generation vaccines are being developed.[15]

ANTIGENICITY AND EPIDEMIOLOGY

Antigenic and Genetic Relationships

The three American equine encephalitides virus complexes, VEE, EEE, and WEE, have been grouped with four additional virus complexes into the *Alphavirus* genus based on their serologic cross-reactivity (Table 12-1).[13] Analysis of structural gene sequences obtained from members of the VEE and EEE virus complexes confirms the antigenic classification and serves as another tool for classifying these viruses (Figure 12-1). The WEE virus complex, including Highlands J, Fort Morgan, and WEE viruses, is identified as recombinant viruses originating from ancestral precursors of EEE and Sindbis viruses and, therefore, falls into a unique genetic grouping of alphaviruses.[36-39]

Venezuelan Equine Encephalitis Virus Complex

The VEE virus complex consists of six closely related subtypes that manifest different characteristics with respect to ecology, epidemiology, and virulence for humans and equines (Table 12-2). The IA/B and C varieties are commonly referred to as epizootic strains. These strains, which have been responsible for extensive epidemics in North, Central, and South America, are highly pathogenic for humans and equines. All epizootic strains are exotic to the United States and have been isolated from areas where virus occurs naturally.[40] Subtypes II, III, IV, V, and VI and varieties ID, IE, and IF are referred to as the enzootic strains.[41-46] Like the epizootic strains, the enzootic strains may cause disease in humans, but they differ from the epizootic strains in their lack of virulence for equines. The enzootic viruses are commonly isolated in specific ecological habitats, where they circulate in transmission cycles primarily involving rodents and *Culex* mosquitoes of the *Melanoconion* subgenus.[47-49] Infection of equines with some enzootic subtypes leads to an immune response capable of protecting the animals from challenge with epizootic strains.[50] Limited data, acquired following laboratory exposures, suggest that cross-protection between epizootic and enzootic strains may be much less pronounced in humans.[51-53]

Eastern Equine Encephalitis Virus Complex

The EEE virus complex consists of viruses in two antigenically distinct forms: (1) the North American and (2) the South American variants.[54] The two forms can be distinguished readily by hemagglutination inhibition and plaque-reduction neutralization tests.[54,55] All North American and Caribbean isolates show a high degree of genetic and antigenic homogeneity. However, they are distinct from the South American and Central American isolates, which tend to be more heterogeneous and form three genetic clades that are readily distinguished from the monophyletic North American EEE viruses.[56,57]

EEE is endemic to focal habitats ranging from southern Canada to northern South America. The virus has been isolated as far west as Michigan but is most common along the eastern coast of the United States between New England and Florida. Enzootic transmission of EEE virus occurs almost exclusively between passerine birds (eg, the perching songbirds) and the mosquito *Culiseta melanura*. Because of the strict ornithophilic feeding behavior of this mosquito, human and equine disease requires the involvement of more general feeders, known as bridging vectors, such as members of the genera *Aedes* and *Coquilletidia*. Mosquito vectors belonging to *Culex* species may play a role in maintaining and transmitting South American EEE strains.[58]

TABLE 12-1

ANTIGENIC CLASSIFICATION OF ALPHAVIRUSES

Antigenic Complex	Virus		
	Species	Subtype	Variety
Western Equine Encephalitis (WEE)	WEE		
	Y 62-33		
	Highlands J		
	Fort Morgan		
	Aura		
	Sindbis	Sindbis	Ockelbo
		Babanki	
		Whataroa	
		Kyzylagach	
Venezuelan Equine Encephalitis (VEE)	VEE	I	A-B
		I	C
		I	D
		I	E
		I	F
		II Everglades	
		III Mucambo	Mucambo
			Tonate
			71D-1252
		IV Pixuna	
		V Cabassou	
		VI AG80-663	
Eastern Equine Encephalitis (EEE)	EEE		North American
			South American
Semliki Forest	Semliki Forest		
	Chikungunya	Chikungunya	Several
		O'nyong-nyong	Igbo ora
	Getah	Getah	
		Sagiyama	
		Ross River	
	Mayaro	Mayaro	
		Una	
Middelburg	Middelburg		
Nduma	Nduma		
Barmah Forest	Barmah Forest		

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Western Equine Encephalitis Virus Complex

Six viruses, WEE, Sindbis, Y 62-63, Aura, Fort Morgan, and Highlands J, comprise the WEE complex. Several antigenic subtypes of WEE virus have been identified, but their geographical distributions overlap.[40] Most of the members of the WEE complex are distributed throughout the Americas, but subtypes of Sindbis virus and its subtypes have strictly Old World distributions.[13] The New World WEE complex viruses can be distinguished readily by neutralization tests. In addition, WEE complex viruses isolated in the western United States (ie, WEE) are antigenically and genetically distinct from those commonly found in the eastern United States (ie, Highlands J).[57,59] Sindbis virus is considered a member of the WEE virus complex based on antigenic relationships. However, sequence comparisons show that WEE, Highlands J, and Fort Morgan viruses are actually derived from a recombination event between ancestral Sindbis and EEE viruses. The structural domains of the recombinant viruses were derived from the Sindbis virus ancestor,

Fig. 12-1. This photograph was taken in 1995 near Buena Vista, Colombia. During large Venezuelan equine encephalitis (VEE) epizootics, typical morbidity rates among unvaccinated equines are 40% to 60%, with at least half of the affected animals progressing to lethal encephalitis. Note the disruption of the ground surface, which is caused by the characteristic flailing or swimming syndromes of moribund animals. Although clinically indistinguishable from the syndromes produced by eastern equine encephalitis and western equine encephalitis viruses, the capability of VEE to initiate explosive and rapidly expanding epizootics makes reliable diagnostic tests essential for the initiation of appropriate veterinary and public health measures.

and the nonstructural domains were derived from the EEE virus ancestor.[57,60]

The most studied member of the WEE virus complex in terms of its epidemiology is the WEE virus itself. The virus is maintained in cycles involving passerine birds and the mosquito *C tarsalis*. Humans (and equines) become involved only tangentially and are considered to be dead-end hosts,[61] indicating that they do not normally contribute to further spread of the virus. Recent studies have isolated WEE virus from male *Ae dorsalis* mosquitoes reared in the laboratory from larvae collected in salt marsh habitats,[62] suggesting that vertical transmission (ie, direct transmission from one generation to the next) in mosquitoes may be an important mechanism for persistence and overwintering in endemic areas.

Epidemiology and Ecology

The evolution of the equine encephalitides in humans is closely tied to the ecology of these viruses in naturally occurring endemic foci. Recent evidence indicates that the relative genetic homogeneity of the EEE and WEE virus complexes may result from the mixing of virus subpopulations as a result of the movement of the virus from one location to another by the

avian hosts. In general, these viruses are maintained in a consistently virulent state, capable of initiating epizootics without development of any significant mutations. In contrast, diversity within the VEE virus complex results from local evolution of these viruses in mammalian hosts that live in defined habitats. Initiation of epizootic and epidemic activity is almost always associated with appearance of significant genetic change.[63]

Human involvement in the form of endemic and epidemic activity occurs most commonly following intrusion into geographical regions where natural transmission cycles are occurring or after perturbation of these cycles by environmental changes or the addition of other vectors.[64] The dramatic exception to this is epizootic VEE, in which the spreading waves of the epizootic among equines can move rapidly over large distances, and humans become infected by mosquitoes that have fed on viremic equines. The high levels of viremia in equines infected with epizootic VEE make them efficient amplifying hosts, with the result that equine infections normally precede human infections by days to weeks.[65] Researchers suggest that it is the adaptation of these viruses for efficient replication in horses that leads to the emergence and efficient epidemic spread of disease.[22,66] Medical personnel should view with some suspicion evidence of widespread human VEE infections outside of endemic areas in the absence of mosquito vectors or in the absence of equine disease; this combination of circumstances may indicate an unnatural release of virus into the environment.

Enzootic VEE virus subtypes, as described above, are maintained efficiently in transmission cycles involving mosquitoes belonging mainly to the subgenus *Melanoconion*. These mosquitoes often live in humid localities with abundant open spaces such as sunny, swampy pastures cut by slowly flowing streams. They are ground feeders, seldom found higher than 8 meters above ground, and prefer feeding on mammals rather than birds.[67] Ground-dwelling rodents, partly because their ecologies are similar to that of the mosquito vectors, are the primary vertebrate hosts for the enzootic forms of VEE virus. After infection, these animals develop viremia of sufficient magnitude and duration to infect mosquitoes feeding on their blood.[68] Other animals, such as bats and certain birds, may play a secondary role.[69] Seroprevalence rates among human populations living in or near endemic VEE areas vary but can approach 100%, suggesting that continuous transmission occurs.[65] However, virus activity within endemic zones can also be highly focal. In one incident at the Fort Sherman Jungle Operations Training Center in the Panama Canal Zone in December 1967, 7 of 12

US soldiers camped in one area developed VEE disease within 2 days, but another group camped only a few yards away showed no disease.[70,71] The incidence of disease during epizootics also varies, but it is often high. During an outbreak in Venezuela, attack rates of 119 per 1,000 inhabitants per month were reported.[72] After an epizootic in Guatemala and El Salvador, overall seroprevalence was estimated at 20%.[73]

Unlike the enzootic strains, the fate of the epizootic strains during interepidemic periods is unclear. The most appealing theory on how epizootic strains arise suggests that they evolve by genetic drift from enzootic strains. Results from oligonucleotide fingerprinting and sequence analysis of I-D isolates from Colombia and Venezuela reveal a close similarity to the epizootic strains, suggesting that the equine virulent epizootic strains arise naturally from variants present in populations of I-D virus.[74,75]

Although the genetic evidence indicates that genetic drift of enzootic strains may lead to the development of epizootic strains, ecological data suggest a strong selective pressure to maintain the enzootic genotype in certain habitats. The enzootic VEE vector *C (Melanoconion) taeniopus* is fully susceptible to both I-AB and I-E strains following intrathoracic inoculation. Orally exposed mosquitoes, however, are fully competent vectors of the enzootic strain, but they fail to develop disseminated infection or transmit epizootic virus.[32,76] In the absence of genetic change, this virus–host interaction appears to be relatively stable. Mosquito resistance to epizootic strains of VEE virus is rare. Epizootic strains have been isolated from a large number of mosquito species, and many have been shown to be efficient vectors.[77] Thus, host switching from enzootic to epizootic vectors may be an important factor in the evolution of epizootic VEE strains. Researchers have suggested that emergence of epizootic strains may result from acquisition of mutations that allow for

TABLE 12-2

THE VENEZUELAN EQUINE ENCEPHALOMYELITIS COMPLEX

Subtype	Variety	Prototype Strain	Origin	Cycle	Disease in Horse	Disease in Man
I	A/B	Trinidad donkey	Donkey (Trinidad)[1]	Epizootic	+	+
	C	P-676	Horse (Venezuela)[2]	Epizootic	+	+
	D	3880	Human (Panama)[3]	Enzootic	−	+
	E	Mena II	Human (Panama)[1]	Enzootic	−	+
	F	78V-3531	Mosquito (Brazil)[4]	Enzootic	−	?
II (Everglades)		Fe3-7c	Mosquito (Florida)[5]	Enzootic	−	+
III (Mucambo)	A	Mucambo (BeAn8)	Monkey (Brazil)[6]	Enzootic	−	+
	B	Tonate (CaAn410-D)	Bird (French Guiana)[7]	Enzootic	−	+
	C	71D-1252	Mosquitoes (Peru)[8]	Enzootic	−	?
IV (Pixuna)		Pixuna (BeAn356445)	Mosquito (Brazil)[6]	Enzootic	−	?
V (Cabassou)		Cabassou	Mosquito (French Guiana)[7]	Enzootic	−	?
VI		AG80-663	Mosquito (Argentina)[9]	Enzootic	−	+

Sources that contain original descriptions of or additional information about this strain: (1) Young NA, Johnson KM. Antigenic variants of Venezuelan equine encephalitis virus: their geographic distribution and epidemiologic significance. *Am J Epidemiol*. 1969;89:286. (2) Walton TE. Virulence properties of Venezuelan equine encephalitis virus serotypes in horses. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus, Washington, DC, 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 134. PAHO Scientific Publication 243. (3) Johnson KM, Shelokov A, Peralta PH, Dammin GJ, Young NA. Recovery of Venezuelan equine encephalomyelitis virus in Panama: a fatal case in man. *Am J Trop Med Hyg*. 1968;17:432–440. (4) Walton TE, Grayson MA. Venezuelan equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 4. Boca Raton, Fla: CRC Press; 1988: 203–231. (5) Chamberlain RW, Sudia WD, Coleman PH, Work TH. Venezuelan equine encephalitis virus from South Florida. *Science*. 1964;145:272. (6) Shope RE, Causey OR, de Andrade AHP, Theiler M. The Venezuelan equine encephalitis complex of group A arthropodborne viruses, including *Mucambo* and *Pixuna* from the Amazon region of Brazil. *Am J Trop Med Hyg*. 1964;13:723. (7) Karabatsos N. *International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates*. 3rd ed. San Antonio, Tex: American Society for Tropical Medicine and Hygiene; 1985. (8) Scherer WF, Anderson K. Antigenic and biological characteristics of Venezuelan encephalitis virus strains including a possible new subtype isolated from the Amazon region of Peru in 1971. *Am J Epidemiol*. 1975;101:356. (9) Contigiani MS, De Basualdo M, Camara A, et al. Presencia de anticuerpos contra el virus de la encefalitis equina Venezolana subtipo VI en pacientes con enfermedad aguda febril. *Revista Argentina de Microbiologia*. 1993;25:212–220.

Adapted with permission from Walton TE, Grayson MA. Venezuelan equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 4. Boca Raton, Fla: CRC Press; 1989: 206.

transmission by abundant equiphilic mosquitoes. More specifically, adaptation to *Ochlerotatus taeniorhynchus* mosquitoes has been a determinant of some recent emergence events, providing further evidence that the ability to switch hosts is critical for emergence of epizootic strains.[66] The introduction of mosquito species into previously unoccupied geographical ranges (eg, *Ae albopictus* into North America) may, therefore, offer the opportunity for epizootic strains to reemerge.

A major epizootic VEE outbreak occurred in the late 1960s and early 1970s. Epizootic virus first reached North America in 1966 but did not reach the United States until 1971. Studies of this epizootic demonstrated that the virus easily invaded territories in which it was formerly unknown,[72] presumably as a result of (*a*) the availability of large numbers of susceptible equine amplifying hosts and (*b*) the presence of competent mosquito vectors. The initial outbreak in North America, and the first recorded such epizootic, occurred in 1966 in Tampico, Mexico, involving approximately 1,000 equines.

By the end of 1969 and the beginning of 1970, the outbreak had expanded to such an extent that the Mexican government requested the TC-83 vaccine from the US Army through the US Department of Agriculture.[78] Despite the vaccination of nearly 1 million equines, the epizootic continued to spread and reached the United States in June 1971. The nature of the virus and the number of human and equine cases prompted the secretary of agriculture to declare a national emergency on July 16, 1971.[79] Subsequent immunization of over 2 million horses and unprecedented mosquito abatement efforts eventually stopped the epizootic before it spread from Texas. Epizootic VEE has not been isolated in the United States since the 1971 outbreak.

The first large outbreak since the 1969–1971 epizootic occurred in 1995 (Figures 12-1 and 12-2). The epizootic began in northwestern Venezuela and spread across the Guajira Peninsula into northeastern Colombia. An estimated 75,000 to 100,000 humans were infected, with over 20 deaths reported. This outbreak

Fig. 12-2. This photograph was taken in 1995 near Maicao, Colombia. Equine vaccination is the most effective means available to prevent Venezuelan equine encephalitis (VEE) epizootics as well as to control emerging outbreaks. Equines are the major amplifying hosts, and maintaining a high rate of immunity in the equine population will largely prevent human infection with the epizootic strains of VEE. Both inactivated and live attenuated vaccines are available for veterinary use, but the ability of the live attenuated vaccine to induce immunity in 7 to 10 days with a single inoculation makes it the only practical vaccination strategy in the face of an outbreak. Other measures used to control outbreaks including using insecticides to reduce mosquito populations and prohibiting the transportation of equines from affected areas.

was caused by an IC strain of VEE virus. By sequence analysis, this strain proved to be essentially identical to a virus that caused an outbreak in Venezuela in 1963.[30] More recently, outbreaks of traditionally enzootic strains of VEE have occurred in Mexico and Central America. Unlike previously identified enzootic strains, these newly emerged strains appear to have increased virulence for humans. Genetic analysis confirms acquisition of mutations, which provides further evidence that emergence of epizootic strains may result from accumulation of genotypic changes in enzootic strains.[80,81]

STRUCTURE AND REPLICATION OF ALPHAVIRUSES

Virion Structure

The alphavirus virion, a spherical particle approximately 60 to 65 nm in diameter, is typically composed of three different structural proteins enclosing a single molecule of single-stranded RNA. The RNA genome is packaged within an icosahedral nucleocapsid, which is constructed from multiple copies of a single species of capsid (C) protein (Figure 12-3). The nucleocapsid is, in turn, surrounded by a lipid envelope derived

from areas of the host cell plasma membrane that had previously been modified by the insertion of two viral glycoproteins. These envelope glycoproteins, E1 and E2, form heterodimers that associate further into trimers[82,83] to form the short spikes on the surface of the virion. The glycoproteins are the primary targets of the neutralizing antibody response and are one of the determinants of tropism and virulence.[84-86] Semliki Forest virus contains a third glycoprotein, E3, which is associated with the E1–E2 dimers on the virion

surface. With other alphaviruses, the E3 protein is shed from the infected cell and does not appear in the mature virion.

Viral Infection

The infection cycle is initiated when the glycoprotein spikes on the virion bind to receptors on the cell surface. The virus is initially localized to coated pits, where it is engulfed in a coated vesicle and transported

Fig. 12-3. Structure of an alphavirus. Shown is the three-dimensional reconstruction of Sindbis virus at 28 Å resolution from computer-processed images taken by electron cryomicroscopy. (**a**) The original electron micrograph shows virus particles in vitreous ice. (**b**) The surface view of the virus shows details of the 80 trimeric spikes, which are arranged in a T=4 icosahedron. Each spike protrudes 50 Å from the virion surface and is believed to be composed of three E1–E2 glycoprotein heterodimers. (**c**) The cross-sectional view shows the outer surface spikes (yellow) and the internal nucleocapsid (blue), composed of the capsid and viral RNA. The space between the spikes and the nucleocapsid would be occupied by the lipid envelope. The green arrows mark visible points of interaction between the nucleocapsid and transmembranal tails of the glycoprotein spikes. (**d**) The reconstructed capsid also exhibits a T=4 icosahedral symmetry. Computer models: Courtesy of Angel M Parades, Cell Research Institute and Department of Microbiology, The University of Texas at Austin, Austin, Texas. Similar but not identical versions of these computer models were published in Paredes AM, Brown DT, Rothnagel R, et al. Three-dimensional structure of a membrane-containing virus. *Proc Natl Acad Sci U S A.* 1993;90:9095–9099.
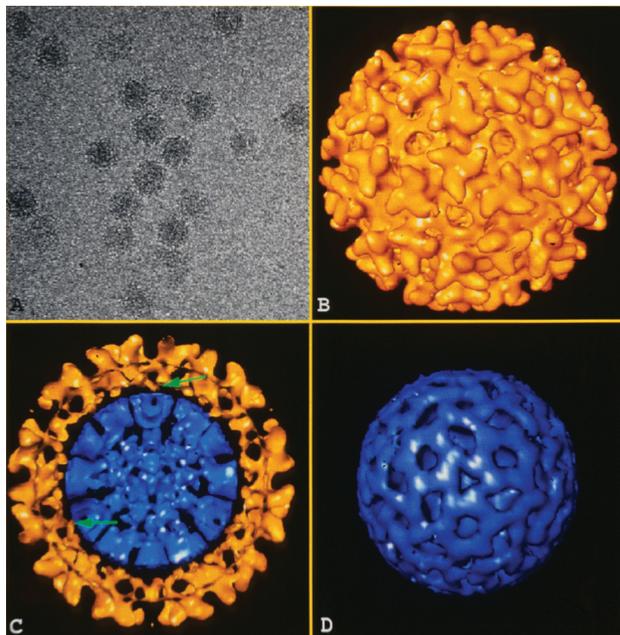
to the endosomal compartment within the interior of the cell. A decrease in the pH in the interior of the vesicle induces a conformational change in the glycoprotein spikes, and rearrangement of the E1 glycoprotein mediates fusion of the virion envelope with the endosomal membrane.[87] This fusion results in the release of the nucleocapsid into the cytoplasm, where disassembly of the nucleocapsid releases the viral RNA genome to the synthetic apparatus of the cell.

Genomic RNA

The viral genome, a positive-stranded RNA of approximately 11,700 nucleotides, has the structural features of messenger RNA (ie, mRNA, a 5′ methylated cap [m7GpppA], and a poly-A tract at the 3′ end).[88] As a complete and functional mRNA, genomic RNA purified from virions is fully infectious when artificially introduced (ie, transfected) into susceptible cells. Similarly, RNA transcribed from a full-length complementary DNA (cDNA) clone of an *Alphavirus* is also infectious, which allows genetic manipulation of these viruses. Mutations introduced into a cDNA clone by site-directed mutagenesis are reflected in the RNA transcribed from the altered clone and in the virus obtained from transfected cells. These procedures are being used to develop improved vaccines,[15] but they could also be used to enhance specific characteristics required for weaponization.

Glycoprotein Synthesis

The *Alphavirus* genome contains two protein coding regions. The 5′ proximal 7,500 nucleotides encode a 220,000-dalton precursor polypeptide, which is proteolytically processed to produce four components of the viral RNA polymerase. The polymerase genes are followed by a second coding region of approximately 3,800 nucleotides, which contains the information that directs the synthesis of the viral structural proteins. Soon after release of the viral genome from the nucleocapsid, the 5′ 7,500 nucleotides of the genome RNA are translated to produce the viral RNA polymerase. Early in infection, the incoming viral genome is also used as a template for the synthesis of a negative-stranded 45S RNA, identical in length to the genome RNA but of opposite polarity. The negative-stranded 45S RNA subsequently serves as a template for the synthesis of additional genomic RNA. The negative-stranded RNA is also used as a template for transcription of a capped and polyadenylated 26S subgenomic mRNA, which is identical to the 3′ third of the genome. The 26S mRNA is translated to yield a precursor polypeptide that is proteolytically processed by cotranslational and post-

translational cleavages to produce the viral structural proteins. The order of the structural proteins within the precursor is C-E3-E2-6K-E1.

As the 26S mRNA is translated, the C protein is produced first and catalyzes its own cleavage from the nascent polypeptide soon after the ribosome transits into the sequences that encode E3. After release of the C protein, the free amino terminus of E3 is bound to the membranes of the rough endoplasmic reticulum. As the synthesis of nascent E3 and E2 continues, the polypeptide is translocated into the lumen of the endoplasmic reticulum, where oligosaccharides and fatty acids are added.[89] A domain of hydrophobic amino acids near the carboxyl terminus of E2 inhibits further transmembranal movement so that the last 30 to 40 amino acids of the E2 polypeptide remain exposed on the cytoplasmic side of the membrane. The 6 K polypeptide probably serves as an signal for membrane insertion of the second glycoprotein, E1, and is subsequently cleaved from both E2 and E1 by signal peptidase.[90] A hydrophobic anchor sequence near the carboxyl terminus of E1 secures the protein in the membrane.

Budding and Release of Progeny Virus Particles

Soon after synthesis, the precursor of E2 (PE2) and E1 interact to form multimeric complexes,[91] which are then transported through the Golgi apparatus, where the final modifications of the oligosaccharide are made. The precursor pE2 is cleaved to the mature E2 and E3 glycoproteins soon after the glycoproteins leave the Golgi apparatus,[92] and the mature viral spikes assume an orientation in the plasma membrane with the bulk of the E2 and E1 polypeptides exposed on the exterior surface of the cell. In vertebrate cells, final assembly of progeny virus particles happens by budding exclusively at the plasma membrane,[93] and in arthropod cells, budding also occurs at intracellular membranes.[94]

In vertebrate cells, budding is initiated when intracellular nucleocapsids bind to the 30– to 40–amino acid cytoplasmic domain of the E2 glycoprotein,[95-97] inducing the formation of a locally ordered array of glycoprotein spikes, which exclude most of the cellular membrane proteins from the region. Additional lateral associations between the individual spikes stabilize the lattice and promote additional E2–C protein interactions. The growing lattice may draw the membrane around the nucleocapsid, completing the process of envelopment with the release of the spherical virus particle. Maximal amounts of virus are typically produced from mammalian cells within 8 to 10 hours after infection, and disintegration of the infected cell is likely caused by programmed cell death (apoptosis) rather than direct effects of the virus on cellular function.[98] In arthropod cells, however, alphaviruses initially replicate to high titer with little or no evidence of cytopathology. The surviving cells continue to produce lesser amounts of virus, often for weeks or months. The ability of the virus to replicate without causing cell death in arthropod cells may be critical for maintenance of the virus in the mosquito vector in nature.

PATHOGENESIS

In humans, the pathogenesis of VEE, EEE, and WEE infections acquired by aerosol, which is the route of greatest biological defense concern, is unknown. Little is known of the pathogenesis even after natural vectorborne infections of humans, mainly because of limited autopsy material. Much of the information on VEE pathogenesis in humans is based on a histological review of 21 human fatalities from the 1962–1963 VEE epidemic in Zulia, Venezuela.[99] With few exceptions, the histopathological lesions in these cases, all among children or young adults, were comparable to those observed in experimentally infected animals. Tissues commonly affected in both humans and animals[100-108] include those of the lymphoid and reticuloendothelial systems as well as the central nervous system (CNS). Widespread hepatocellular degeneration and interstitial pneumonia, not ordinarily seen in experimentally infected animals, were frequent histological findings in these cases of severe human disease. Much of the understanding of the pathogenesis of VEE, EEE, and WEE has relied on animal studies. However, little work has been done in recent years with EEE and WEE viruses, and animal models have failed to recapitulate important characteristics of the human conditions. Recently, a hamster model for EEE, which appears to more closely resemble human EEE, has been developed and appears promising.[109] The pathogenesis of VEE virus infection, in contrast, has been extensively studied in animals, and the remainder of this section covers that subject.

The clinical and pathological responses of the host to VEE infection are highly dependent on a number of host and viral factors, including

- the species, immune status, and age of the host;
- the route of infection; and
- the strain and dose of virus.

Most of the existing experimental data have come from studies using rodent models challenged with the virulent Trinidad donkey (TrD) strain of VEE, an epizootic IA serotype virus, or its genetic clone V3000. A few nonhuman primate studies with monkeys have also been done. In animal models, as in humans, the lymphatic system and the CNS are consistent target organs. However, the relative degree of injury caused to these tissues varies. Virulent VEE virus causes limited and reversible lesions to the lymphoid organs of mice and nonhuman primates,[101-105] but in guinea pigs and hamsters, it causes extreme and irreversible damage to those organs.[106,107] As a result, in the guinea pig and hamster models, death occurs before the development of serious CNS disease.[103,104] The host species and the route of administration of VEE virus greatly affect CNS disease development. Mice uniformly exhibit a severe paralytic episode before death from diffuse encephalomyelitis following peripheral or aerosol administration of TrD or V3000.[101,105,110,111] Nonhuman primates, however, reportedly exhibit few if any clinical signs of encephalitis following peripheral inoculation with TrD, and only modest perivascular cuffing and gliosis, mainly in the thalamus, hypothalamus, and olfactory areas of the brain.[100] Monkeys infected intranasally had more moderate inflammation, especially in the cortex and hypothalamus,[112] yet a Colombian epizootic strain of VEE given by the aerosol route caused severe clinical and pathological CNS signs and resulted in death in approximately 35% of rhesus monkeys.[102] Both mice and cynomolgus monkeys challenged intracerebrally with TrD or related VEE strains developed severe and lethal neurological signs with moderate to severe brain histopathology.[112,113]

The mechanisms of neuroinvasion by VEE virus represent an important issue, particularly regarding immunoprophylaxis. The specific mechanism of neuroinvasion in the case of peripheral inoculation of virus is not completely understood, yet animal studies have elucidated some important features. In mice inoculated peripherally and subsequent to the development of viremia, virulent VEE virus is detectable in the brain, initially in the olfactory bulbs, and usually within 48 hours of infection.[111,114,115] It appears that virus in the blood escapes from fenestrated capillaries supplying the olfactory lining of the nasal tract. Virus may then invade olfactory neuron cell bodies or their axons and may be carried via the olfactory nerves into the olfactory bulbs of the brain. Surgical or chemical ablation of the olfactory lining in mice reportedly delayed neuroinvasion via the olfactory nerves.[114] An alternative theory, direct invasion of the brain across the blood-brain barrier,[104,116] seems less compelling than the olfactory route.

The understanding of the mechanism of neuroinvasion after respiratory infection is more clear. An early and strong target of virulent VEE virus administered by aerosol has been shown to be the olfactory neuron.[111] This cell type, a so-called "bipolar neuron," is in direct contact with inspired air at one pole and synapses with resident neurons in the olfactory bulb at the opposite pole, offering a direct connection to the brain independent of the development of viremia. In mice, both the nasal olfactory epithelium and the olfactory nerve axon
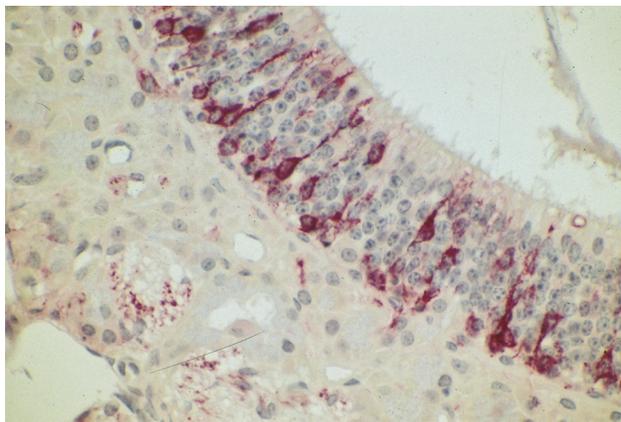
Fig. 12-4. Nasal tissue, BALB/c mouse, 2 days after exposure to aerosolized Venezuelan equine encephalitis (VEE) virus. Note immunoreactive olfactory epithelium and olfactory nerves. Alkaline phosphatase-labeled streptavidin method using rabbit antiserum to VEE virus (Mayer's hematoxylin counterstain, original magnification x 300).
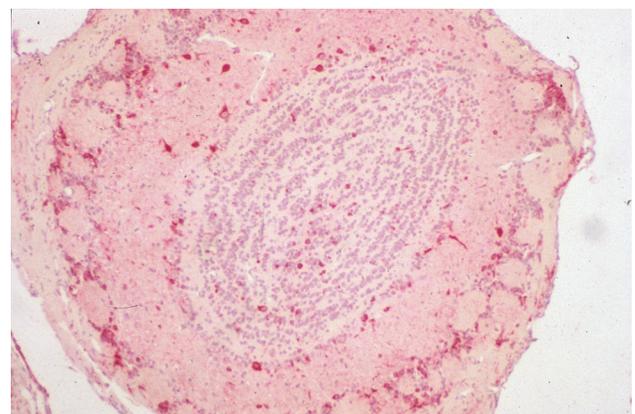
Fig. 12-5. Olfactory bulb, BALB/c mouse, 2 days after exposure to aerosolized Venezuelan equine encephalitis (VEE) virus. Note immunoreactive cells. Alkaline phosphatase-labeled streptavidin method using rabbit antiserum to VEE virus (Mayer's hematoxylin counterstain, original magnification x 150).

bundles in the underlying connective tissue exhibit VEE virus antigen within 24 hours of aerosol infection (Figure 12-4), and the olfactory bulbs show viral infection shortly thereafter (Figure 12-5). In rhesus monkeys inoculated intranasally with VEE virus, the virus also gains access to the olfactory bulb within 24 hours after infection and before the onset of viremia, suggesting direct neuroinvasion via olfactory neurons similar to neuroinvasion in the mouse.[117] However, in inoculated monkeys whose olfactory nerves had been surgically removed, VEE virus was still able to reach the olfactory bulb by 36 hours after infection, presumably by the vascular route. Although the olfactory bulb and olfactory tract were sites of early viral replication, the virus did not appear to spread to the rest of the brain along the neural tracts in these monkeys, as it does in mice. The teeth are another early target of VEE administered peripherally or by aerosol,[110,111,114] and the trigeminal nerves appear to carry VEE virus from the teeth into the brain as an alternate, although probably less significant, route of neuroinvasion. The mechanisms of neuroinvasion by peripheral versus aerosol administration are of significant practical concern because, as studies have shown, the immunological mechanisms of virus neutralization respective to each route can vary greatly.[118-120] The efficiency and rapidity of neuroinvasion after aerosol infection also place high demands on the vaccines used for immunoprophylaxis (vaccines are discussed later in this chapter). Neurons are the primary viral target in the brain and neuronal death by necrosis and/or apoptosis, accompanied by inflammatory changes, are the key consequences of infection.[99-102,110,111]

CLINICAL DISEASE AND DIAGNOSIS

The three equine encephalomyelitis virus complexes within the *Alphavirus* genus, EEE, WEE, and VEE, are also recognized for their potential for neuroinvasion and encephalitis in humans, sometimes in epidemic proportions. However, many of the infections caused by these viruses are manifested as systemic viral febrile syndromes, and infections by EEE and WEE viruses may remain subclinical. Furthermore, these alphaviruses vary markedly in both their neurotropism and the severity of their neurological sequelae. Depending on the virus, patients presenting with the general syndrome of *Alphavirus* encephalitis have a varying combination of fever, headache, confusion, dysphasia, seizures, paresis, ataxia, myoclonus, and cranial nerve palsies.

Venezuelan Equine Encephalitis

The IA, IB, and IC variants of VEE virus are pathogenic for equines and have the capacity for explosive epizootics with epidemic human disease. Epidemics of VEE affecting 20,000 to 30,000 people or more have been documented in Venezuela and Ecuador. In contrast to the other *Alphavirus* encephalitides (EEE and WEE), epizootic strains of VEE are mainly amplified in equines, rather than birds, so that equine disease normally occurs before reports of human disease. Enzootic VEE strains (variants ID, IE, and IF and subtypes II, III, IV, V, and VI) are not recognized as virulent for equines, but disease has been documented with most of these variants in humans who reside in or move into enzootic foci, or after laboratory infections (see Table 12-2). The resulting syndromes appear to be similar, if not indistinguishable, from the syndrome produced by epizootic variants, which ranges from undifferentiated febrile illness to fatal encephalitis. In nonhuman primates, aerosol exposure to enzootic strains results in a febrile illness with indications of encephalitis virtually indistinguishable from that seen with epizootic strains in terms of onset, severity, and duration.[121]

After an incubation period that can be as short as 28 hours but is usually 2 to 6 days, patients typically develop a prostrating syndrome of chills, high fever (38°C–40.5°C), headache, and malaise.[122] Photophobia, sore throat, myalgias, and vomiting are also common symptoms. Frequent signs noted on physical examination include conjunctival injection, erythematous pharynx, and muscle tenderness. Although essentially all human infections with VEE virus are symptomatic,[70,71] only a small percentage manifest neurological involvement.[123] In one epidemic, the ratio of encephalitis to infections was estimated at less than 0.5% in adults, although possibly as high as 4% in children.[124] Mild CNS involvement is evidenced by lethargy, somnolence, or mild confusion, with or without nuchal rigidity.[8] Seizures, ataxia, paralysis, or coma indicate more severe CNS involvement. In children with overt encephalitis, case fatalities may be as high as 35%, compared with 10% for adults.[125] However, for those who survive encephalitic involvement, neurological recovery is usually complete,[126] although one report documented motor disorders and an increased incidence of seizures in children after VEE outbreaks.[126] Abortions and increased fetal deaths have also been attributed to VEE virus infection.[30,127] School-aged children are believed to be more susceptible to a fulminant form of disease, which follows a lethal course over 48 to 72 hours in which depletion of lymphoid tissues is prominent.[99,128,129]

In the first 3 days of illness, leukopenia and elevated serum glutamic-oxaloacetic transaminase are common.

For those with CNS involvement, a lymphocytic pleocytosis of up to 500 cells per *μl* can be observed in the cerebrospinal fluid (CSF). The CSF pleocytosis may be acutely polymorphonuclear but soon becomes predominantly lymphocytic.

Specific diagnosis of VEE can be accomplished by virus isolation, serologic testing, or both.[130] During the first 1 to 3 days of symptoms of nonspecific febrile illness, VEE virus may be recovered from either the serum or the nasopharynx.[131] Despite the theoretical possibility of person-to-person transmission of virus present in the nasopharynx, no such occurrences have been reported. Identification of the VEE subtype of an isolate involved can be accomplished by cross-neutralization tests. In nonhuman primates, the virus is found in the blood for the first 2 to 3 days after aerosol exposure, but levels are low compared to what has been reported for natural infection and may not be detectable after fever onset for enzootic strains.[121,132] VEE virus can be isolated from the nasopharynx of nonhuman primates for up to 5 days after aerosol exposure of naïve animals. Hemagglutination inhibition, enzyme-linked immunosorbent assay (ELISA), or plaque-reduction neutralization antibodies appear as viremia diminishes. Complement-fixing antibodies make their appearance later during convalescence. VEE IgM antibodies are present in acute phase sera,[71] and VEE IgM tests reportedly do not react with sera from patients with EEE or WEE.[133] Because patients with encephalitis typically come to evaluation later in the course of clinical illness, virus is recovered less often from them,[132] and they usually have serum antibody by the time of clinical presentation.[134] Immunity after infection is probably lifelong to the homologous serotype, but cross-immunity may be weak or nonexistent to heterologous serotypes.[51-53] Thus, when viewed either as an endemic disease threat or as a potential biological warfare threat, adequate immunization will require polyvalent vaccines.

Eastern Equine Encephalitis

EEE is maintained in a natural transmission cycle between *Culiseta melanura* mosquitoes and passerine birds in swampy and forested areas. EEE outbreaks are typically recognized when severe equine or human encephalitis occurs near such areas.[135] During vectorborne EEE epidemics, the incidence of human infection is low (< 3% of the population at risk), and the neurological attack rate in one outbreak was estimated at 1 in every 23 cases of human infection.[136,137] However, the effect on morbidity and mortality of aerosol-acquired EEE infection (the expected route of infection in a biological warfare offensive) in humans is unknown, although animal studies indicate that EEE

by aerosol is lethal.[138] The incubation period in humans varies from 5 to 15 days. Adults typically exhibit a febrile prodrome for up to 11 days before the onset of neurological disease[139]; however, illness in children exhibits a more sudden onset.[140] In natural outbreaks, viremia occurs during the febrile prodrome,[141] but is usually undetectable by the time clinical encephalitis develops, when hemagglutination inhibition and neutralizing antibodies become evident.[142] Despite the development of a prompt and neutralizing humoral response, the virus is not eliminated from the CNS, and progressive neuronal destruction and inflammation continue.

EEE is the most severe of the arboviral encephalitides, with high mortality and severe neurological sequelae.[143] During EEE outbreaks, the attack, morbidity, and fatality rates are highest in young children[144] and elderly persons.[145] Case fatality rates are estimated at 50% to 75%, but asymptomatic infections and milder clinical illness are underreported. The illness is characterized by rapid onset of high fever, vomiting, stiff neck, and drowsiness. Children frequently manifest generalized, facial, or periorbital edema. Motor involvement with paresis is common during the acute phase. Major disturbances of autonomic function, such as impaired respiratory regulation or excess salivation, may dominate the clinical picture. Between 30% and 70% of survivors have long-term neurological sequelae such as seizures, spastic paralysis, and cranial neuropathies. Cognitive impairment ranges from minimal brain dysfunction to severe dementia.

Clinical laboratory findings in patients with EEE often demonstrate an early leukopenia followed by a leukocytosis. Elevated opening pressure is commonly noted on lumbar puncture and, especially in children, the CSF lymphocytic pleocytosis may reach a cell count of thousands of mononuclear cells per microliter. Specific diagnosis of EEE depends on virus isolation or serologic testing in which rising titers of hemagglutination inhibition, complement-fixing, or neutralizing antibodies are observed. IgM antibodies are usually detectable in acute-phase sera.[133] As with other alphaviruses, neutralization tests are the most specific. Immunohistochemistry can also be performed postmortem on fixed brain samples. In nonhuman primates exposed by aerosol to EEE, the period from fever onset until the animal is moribund is less than 48 hours regardless of dose.[138]

Western Equine Encephalitis

Like VEE, WEE (by mosquito bite) is less virulent for adult humans than for equines and children, with lower rates of fatalities and neurological sequelae.[146]

As with EEE, infants and elderly persons are especially susceptible to severe clinical illness and neurological sequelae, with case fatality rates of about 10%. Highlands J virus, an antigenically related member of the WEE complex that is isolated frequently in the eastern United States, rarely infects humans.

The incubation period is 5 to 10 days for natural infection. By aerosol, in nonhuman primates, the incubation period is 4 to 5 days.[138] A large percentage of patients with vectorborne infections are either asymptomatic or present with a nonspecific febrile illness or aseptic meningitis. The ratio of encephalitis cases per infection has been estimated to vary from 1 per 1,150 in adults, to 1 per 58 in children, to 1 per 1 in infants.[64] However, the severity of the syndrome and the incidence of inapparent infection almost certainly depend on the strain and dose of virus, and the route of infection. Some unusual isolates show high virulence in laboratory animals,[147] and in one study of laboratory-acquired infections in adults, two of five patients died.[148] Symptoms usually begin with malaise, headache, and fever, followed by nausea and vomiting.[149] Telemetry data from nonhuman primates aerosol exposed to WEE found, in addition to fever, increases in heart rate and changes in electrocardiograph recordings, indicative of sinus tachycardia.[150,151] A transient leukopenia followed by a pronounced leukocytosis composed almost entirely of segmented neutrophils correlated with a poor prognosis. Fever severity also correlated with a poor prognosis. Over the next few days, the symptoms intensify, and somnolence or delirium may progress into coma. The severity of neurological involvement is inversely related to age, with over 90% of children younger than 1 year exhibiting focal or generalized seizures.[152] Physical examination typically reveals nuchal rigidity, impaired sensorium, and upper motor neuron deficits with pathologically abnormal reflexes.

Patients with the most severe infections usually die within the first week of clinical illness, with overall case fatalities averaging 10%. Other patients begin a gradual convalescence after the first week of encephalitic symptoms. Most adults recover completely, but it may take months to years to recuperate from fatigability, recurrent headaches, emotional lability, and impaired concentration.[153] Some patients have permanent residua of motor weakness, cognitive deficits, or a seizure disorder. Children carry a higher incidence of neurological sequelae, ranging from less than 1% in those older than 1 year, to 10% in infants 2- to 3-months old, to more than 50% in newborns. Congenital infection in the last trimester of pregnancy has been described, with resultant encephalitis in the infants.[154] In nonhuman primates, aerosol exposure to a dose equivalent to 10 times the median infective dose produced fever, and 50% of the animals developed clinical signs indicative of encephalitis. Twenty-five percent of those animals died from the infection by day 9 postexposure.[150]

Viremia is rarely detectable by the time patients present with encephalitic symptoms, but IgM, hemagglutination inhibition, and neutralizing antibodies can generally be found by the end of the first week of illness, and they increase in titer during the next week.[133,155,156] In nonhuman primates exposed to aerosolized WEE, the virus was not detectable in the serum or nasopharynx postexposure.[150] Low levels of virus were seen in spinal taps. Antibody response by ELISA or in-vitro neutralization was not detectable until day 9 postexposure, after animals had already died from the infection. Complement-fixing serologic responses generally appear in the second week and rise thereafter. Isolation of virus with up to a 4-fold increase in titer is diagnostic, but because of serologic cross-reactions with other alphaviruses, neutralization tests are preferred. Examination of the CSF reveals a lymphocytic pleocytosis ranging from 10 to 400 mononuclear cells per microliter. WEE virus may occasionally be isolated from the CSF taken within the first 2 days of fever, and is frequently recovered from brain tissue on postmortem examination.[157] Natural infection presumably confers long-term immunity; however, it may not protect against aerosol exposure.[158]

Differential Diagnosis of Alphavirus Encephalitis

Most acute infections with VEE and WEE produce a moderately severe but nonspecific clinical illness, consisting of fever, headache, and myalgias. Therefore, in a potential biological warfare scenario, alphaviruses should be considered in the differential diagnosis whenever epidemic febrile illness occurs, especially if several patients progress to neurological disease. Sick or dying equines near an epidemic febrile illness among troops should immediately suggest the possibility of large-scale *Alphavirus* exposure. Other potential biowarfare agents that may infrequently produce or imitate a meningoencephalitic syndrome include *Brucella* species, *Yersinia pestis*, *Salmonella typhi*, *Coxiella burnetii*, and *Clostridium botulinum*. As with any diagnosis of meningoencephalitis, it is imperative to rule out any potential cause that may be specifically treatable.

For encephalitis cases that are more sporadic in their occurrence, other important viral etiologies that might not be readily discriminated from the alphaviruses by clinical features are listed in Table 12-3. This list is not all-inclusive but suggests other viral encephalitides

TABLE 12-3

SOME IMPORTANT VIRAL CAUSES* OF ENDEMIC ENCEPHALOMYELITIS

Virus *Family*	Genus	Species
Togaviridae	*Alphavirus*	Eastern equine
		Western equine
		Venezuelan equine
Flaviviridae		St. Louis
		Murray Valley
		West Nile
		Japanese
		Dengue
		Tick-borne complex
Bunyaviridae		LaCrosse
		Rift Valley
		Toscana
Paramyxoviridae	*Paramyxovirus*	Mumps
	Morbillivirus	Measles
	Henipavirus	Hendra
		Nipah
Arenaviridae	*Arenavirus*	Lymphocytic choriomeningitis
		Machupo
		Junin
Picornaviridae	*Enterovirus*	Poliovirus
		Coxsackievirus
		Echovirus
Reoviridae		Colorado tick fever
Rhabdoviridae	*Lyssavirus*	Australian bat lyssavirus
		Rabies
Herpesviridae	*Herpesvirus*	Herpes simplex virus types 1 and 2
		Epstein-Barr virus
		Cytomegalovirus

*Not all-inclusive

that should be considered if a patient presents, *a priori*, with an encephalitic syndrome. Epidemiological, historical, and laboratory information are critical to differential diagnosis. Immediate and careful consideration must be given to treatable infections that may mimic viral encephalitis (Exhibit 12-1), because prompt and appropriate intervention can be lifesaving. In addition, vascular, autoimmune, and neoplastic diseases may imitate infectious meningoencephalitis.

For endemic meningoencephalitic disease that occurs outside biowarfare theaters, the geographical locale and the patient's travel history are of preeminent importance in diagnosing an arboviral encephalitis. Risk for disease is increased relative to the patient's amount of arthropod contact near swampy or for-

ested areas during the summer. Encephalitic illness of equines in the surrounding locale is an important indication of ongoing transmission of encephalitic alphaviruses. Animal studies have indicated that virus may not be detectable in the serum during the febrile period, and antibody responses may be weak or nonexistent, making diagnosis difficult, which is particularly true for WEE. Examination of the CSF, including viral cultures, is critical in differentiating bacterial from viral infections, and infectious from noninfectious etiologies. Serum and CSF tests based on polymerase chain reaction techniques hold great promise in more rapid diagnosis of infectious encephalitis. In some instances it will be necessary to (*a*) institute therapy for possible, treatable, infecting organisms and (*b*) await definitive laboratory diagnostic tests.

Medical Management and Prevention

No specific therapy exists for the togaviral encephalitides; therefore, treatment is aimed at management of specific symptoms (eg, anticonvulsant medication and airway protection). The extremes of high fever occasionally produced by WEE infection in humans

EXHIBIT 12-1

NONVIRAL CAUSES OF ENCEPHALOMYELITIS

Treatable infectious conditions that can mimic viral encephalitis:
  Partially treated bacterial meningitis
  Brain abscess
  Subdural empyema
  Embolic encephalitis associated with bacterial endocarditis
  Lyme disease
  Tuberculous meningitis
  Fungal meningitis
  Rocky Mountain spotted fever
  Cat scratch disease
  Cerebral malaria
  Trypanosomiasis
  Toxoplasmosis

Vascular, autoimmune, and neoplastic diseases that can mimic infectious meningoencephalitis:
  Lupus cerebritis
  Cerebral and granulomatous arteritis
  Lymphomatous cerebritis
  Whipple's disease
  Behçet syndrome
  Carcinomatosis meningitis

are a special problem among the arboviral encephalitides that may require aggressive antihyperthermia measures. The US Army has extensive experience with a live attenuated vaccine for VEE (TC-83) in humans. However, this vaccine is expected to protect efficiently against only IA/B and IC serotypes. The TC-83 vaccine is also reactogenic, with over 20% of vaccine recipients experiencing fever, malaise, and headache after the vaccination. Half of these patients experience symptoms severe enough to warrant bed rest for 1 to 2 days.

Use of an effective vaccine in horses would prevent outbreaks of epizootic VEE, because equines are the major amplifying species for VEE virus. Vaccination of horses is not a useful public health tool for EEE, WEE, or enzootic VEE, however, because horses are not important as amplifying hosts for these diseases. Investigational formalin-inactivated vaccines for humans are available for WEE and EEE, but they require multiple injections and are poorly immunogenic. Integrated mosquito control measures also have significant impact on ameliorating epidemic transmission.

IMMUNOPROPHYLAXIS

Relevant Immune Effector Mechanisms

The equine encephalomyelitis viruses constitute both an endemic disease threat and a biological warfare threat; therefore, adequate immunoprophylaxis of military personnel will require protection against both vectorborne and aerosol-acquired infections. The requirements for protection against parenteral infection are well described, but the requirements for protection against infectious aerosols are more stringent and are largely unidentified. Within a few days of infection with an *Alphavirus*, specific antibodies can be detected in the serum of animals or humans. Within 7 to 14 days, a virus-neutralizing antibody response develops, as measured by the ability of serum antibodies to block virus infectivity in vitro or in vivo. Protection from mosquito-vectored *Alphavirus* disease is believed to be primarily mediated by this virus-specific neutralizing antibody response, which is largely directed against epitopes on the E2 glycoprotein. Protection mediated by nonneutralizing antibodies to alphaviruses, directed mainly at epitopes on the E1 glycoprotein, has also been described.[159-161] In nonhuman primates and mice, protection from aerosol exposure correlated with serum neutralization or antibody titers.[120,132,162,163]

Other nonspecific immune responses that occur following *Alphavirus* infection include the induction of interferon (IFN)[164-167] and the activation of cytotoxic macrophages.[168] Several studies have demonstrated the importance of the innate immune response, specifically IFN-α, in resistance to *Alphavirus* infection. Studies with Semliki Forest virus and VEE virus have shown that IFN α/ βR knockout mice are more susceptible to infection.[169-171] Pre- and postexposure administration of IFN or inducers of IFN in vivo may be effective for protection against alphaviruses.[172,173] IFN-β was beneficial in protection against the Semliki Forest virus peripheral challenge when administered up to 6 days postexposure. Mice were resistant to subcutaneous challenge with the TrD strain of VEE virus and were partially protected from inhalation challenge when administered pegylated IFN-α on days -2 and +5.[174] Pretreating mice with poly IC afforded partial protection against peripheral challenge with EEE virus,[169] and poly-ICLC similarly induces protection against respiratory challenge with WEE virus.[173] Although these studies clearly indicate the importance of IFNs in host resistance to *Alphavirus* infections, further study is necessary to determine the efficacy of IFN-α for prophylactic or therapeutic use in humans. There have also been reports of virus-specific cytotoxic T-cell responses induced against alphaviruses,[175-178] although it has proven difficult to show that these T-cell responses play a significant role in protection.

Passive Immunization

Passive transfer of neutralizing antisera or monoclonal antibodies to naive recipients protects animals from subsequent parenteral challenge with homologous VEE strains.[160,167,179] Passive transfer of nonneutralizing, anti-E1 monoclonal antibodies directed against appropriate epitopes is also protective against Sindbis,[159] WEE,[161] and VEE[160] viruses. However, for the respiratory route of infection, uniform protection was not observed after passive transfer of hyperimmune serum to hamsters[161] or neutralizing monoclonal antibodies to mice,[180] suggesting that either additional immune mechanisms or the presence of protective antibodies along the respiratory tract may be needed. The time between the administration of immune serum and virus exposure may also be relevant. Protection of mice from intracerebral inoculation with WEE virus was observed if immune serum was given no more than 3 days before virus exposure.[181,182] Similarly, monkeys passively immunized with horse antiserum to EEE or WEE resisted intranasal challenge from homologous virus 24 hours later, but they were unable to resist a second challenge with the same virus 7 weeks later.[183] However, as the immune serum given in both studies was xenogeneic, the loss of protective capacity was

presumably related, in part, to active clearance of the immune serum by the recipients.

The effect of administering immune serum to animals after the establishment of intracerebral infections has also been evaluated. Several studies, using different alphaviruses, demonstrated at least partial protection if the immune serum was administered within 24 hours of infection.[181,182,184-186] Other researchers have suggested that postinfection serum transfer may also cause a more severe pathology, or may merely delay the onset of disease symptoms.[187] Aggressive serotherapy following infections of two laboratory workers who developed acute WEE encephalitis resulted in the survival of one patient[188] but was ineffective in the second patient.[183]

In an EEE outbreak in New Jersey in 1959, 22 of 32 diagnosed patients died. Most patients had demonstrable antibody during the onset or progression of encephalitis, and neutralizing antibody titers in sera from patients who died were generally similar to those observed in patients who recovered.[189] This finding, coupled with animal studies indicating that transfer of virus-neutralizing anti-sera was unable to prevent progression of disease if infection of the brain was firmly established as described above, indicates that serotherapy would be an ineffective means of treatment for these virus infections, unless initiated early in the course of disease.

Active Immunization

Vaccines available for use against the equine encephalomyelitis viruses include TC-83, which is a live attenuated vaccine for VEE, and inactivated vaccines for VEE, EEE, and WEE. All these vaccines are used under the Food and Drug Administration's investigational new drug status. The characteristics of these vaccines and the responses induced in human vaccinees are summarized in Table 12-4.

Live Vaccines

The TC-83 VEE vaccine, which was developed in 1961 by serial passage of the virulent TrD strain in fetal guinea pig heart cells,[190] is administered subcutaneously (0.5 mL) at 1×10^4 to 2×10^4 plaque-forming units per dose. The vaccine was used initially in laboratory and field personnel at risk for exposure to VEE,[191] and over 6,000 people received the vaccine between 1964 and 1972.[191] For reasons that remain unclear, approximately 20% of the people who receive TC-83 fail to make a minimum neutralizing antibody response and probably would not be protected should they be exposed to the virus. Another 25% of vaccine recipients experience clinical reactions ranging from mild transient symptoms to fever, chills, sore throat,

TABLE 12-4

VACCINES AVAILABLE FOR VEE, EEE, AND WEE VIRUSES

Vaccine	Form/Strain	Dose (mL)/ Route of Administration	Responding Schedule	Booster Dose/%	Duration[*]	Route
VEE (TC-83) Attenuated	TrD	0.5 mL/sc	Day 0	82%	92%	C-84/sc
VEE (C-84)[†]	Inactivated TC-83	0.5 mL/sc	After TC-83	76% NR[‡] 100% WT[§]	60% 100%	0.5 mL/sc
EEE	Inactivated PE-6[¥]	0.5 mL/sc	Days 0, 28	58%	75%	0.1 mL/id
WEE	Inactivated CM-4884[¥]	0.5 mL/sc	Days 0, 7, 28	50%	20%	0.5 mL/sc

[*]% of responders whose virus-neutralizing titers persist for at least 1 year
[†]current IND protocols specify use of C-84 only as a booster vaccine
[‡]TC-83 nonresponders
[§]TC-83 responders given C-84 to boost waning titers
[¥]laboratory designation
EEE: Eastern equine encephalitis
id: intradermal
IND: investigational new drug
sc: subcutaneous
TC: cell culture
TrD: Trinidad donkey
VEE: Venezuelan equine encephalitis
WEE: Western equine encephalitis

and malaise sufficient to require bed rest.[192] However, for recipients who respond with postvaccination titers of at least 1 per 20, long-term follow-up studies have shown that titers persist for several years.[193] In humans, documented vaccine-breakthrough infections have been attributed largely to exposure to heterologous, enzootic strains of VEE virus.[51-53] Although pregnant mares were not adversely affected by TC-83,[194] pregnant women are advised not to receive the TC-83 vaccine, because wild-type VEE may have been associated with spontaneous abortions or stillbirths during an epidemic in Venezuela in 1962.[100]

In animals, TC-83 vaccination will protect hamsters from a lethal VEE subcutaneous or aerosol challenge,[162] although up to 20% of hamsters may die of vaccine reactions.[106,195] Subcutaneous vaccination of monkeys[112] with the vaccine produces neutralizing antibody responses in serum and protection from virulent VEE virus delivered by peripheral or intranasal challenge. However, TC-83 provides only partial protection against aerosol challenge in outbred mice.[116] TC-83 has been extensively administered to horses, burros, and mules, in part because large numbers of equines were vaccinated during the 1969–1970 epizootic. TC-83 vaccination produces febrile responses and leukopenia in some equines,[196,197] but neutralizing antibody responses to homologous (serotype IA) virus eventually develop in 90% of these animals.[196,198] Although it was difficult to accurately assess vaccine efficacy under the conditions of an ongoing epizootic, herds of animals known to have been vaccinated at least 2 weeks before any disease occurrence in the area did not sustain any VEE-related deaths, whereas unimmunized herds experienced up to 60% mortality rates.[192]

The phenomenon of vaccine interference, in which prior immunity to heterologous alphaviruses inhibits vaccine viral replication and subsequent immune responses, is an unresolved problem with the use of TC-83 and presumably with other live attenuated alphavirus vaccines. This occurrence has been observed in horses,[199,200] in which preexisting antibodies to EEE and WEE may have interfered with TC-83 vaccination. Interference has also been observed in humans, in which preexisting immunity to a live *Alphavirus* vaccine inhibited effective vaccination with a second, different *Alphavirus* vaccine.[201]

Inactivated Vaccines

Against VEE (C-84). Early attempts to develop an inactivated vaccine against VEE resulted in preparations that contained residual live virus and caused disease in 4% of those who received it.[184,202] Develop- ment of an inactivated VEE vaccine (C-84) was begun, using the TC-83 attenuated strain of virus, because of the problems associated with incomplete inactivation.[203] Initial clinical trials with the C-84 inactivated vaccine were begun in 1976 in 14 volunteers previously vaccinated with TC-83, and subsequently in 14 naive volunteers.[204] The vaccine was found to be safe and elicited only mild tenderness at the injection site. Although C-84 was immunogenic, three doses were required to maintain neutralizing antibody titers in recipients. A subsequent study has shown that most of the TC-83 nonresponders and all of the individuals with waning titers responded to a booster dose of C-84 with a high probability of maintaining a titer for 3 years.[191] However, the observation that hamsters given C-84 vaccine were protected from subcutaneous challenge but not from an aerosol exposure to VEE virus[162] raised concerns that C-84 vaccination may not protect at-risk laboratory workers from aerosol exposure. Therefore, C-84 is currently administered only as a booster immunogen.

Against EEE and WEE. The PE-6 strain of EEE virus was passed in primary chick-embryo cell cultures, and then it was formalin-treated and lyophilized to produce an inactivated vaccine for EEE.[205] This vaccine is administered as a 0.5-mL dose subcutaneously on days 0 and 28, with 0.1-mL intradermal booster doses given as needed to maintain neutralizing antibody titers. In initial clinical trials, mild reactions to the vaccine were observed, and immunogenicity was demonstrated.[206] The vaccine was given to 896 at-risk laboratory workers between 1976 and 1991. No significant clinical reactions were observed. A long-term follow-up study of 573 recipients indicated a 58% response rate after the primary series, and a 25% chance of failing to maintain adequate titers for 1 year. Response rates and persistence of titers increased with the administration of additional booster doses.[191]

The WEE vaccine was similarly prepared using the B-11 or CM-4884 virus strain, and it caused only mild clinical reactions when administered to WEE-naive individuals.[207] Between 1976 and 1990, 359 laboratory workers were vaccinated with the WEE vaccine. Long-term follow-up studies have indicated that administration of three doses of 0.5 mL subcutaneously on days 0, 7, and 28 results in a 50% response rate (neutralization titer > 1:40) after the primary series. Only 20% of the recipients maintain a titer for 1 year, although this level can be increased to 60% to 70% with additional booster doses.[207]

Active programs are ongoing in a variety of government and university laboratories to develop safe and effective vaccine alternatives.

SUMMARY

The equine encephalomyelitis viruses consist of three antigenically related viruses within the *Alphavirus* genus of the family *Togaviridae*: (1) VEE, (2) WEE, and (3) EEE. These viruses, which are vectored in nature by various species of mosquitoes, cause periodic epizootics among equines. Infection of equines with virulent strains of any these viruses produces a similar clinical course of severe encephalitis with high mortality. However, the clinical course after infection of humans differs. EEE is the most severe of the arbovirus encephalitides, with case fatality rates of 50% to 70%. WEE virus is generally less virulent for adults, but the infection commonly produces severe encephalitis in children, with case fatality rates approaching 10%. In contrast, encephalitis is rare after VEE virus infection, but essentially all infected individuals develop a prostrating syndrome of high fever, headache, malaise, and prolonged convalescence.

Although natural infections are acquired by mosquito bite, these viruses are also highly infectious in low doses as aerosols. These viruses, which can be produced in large amounts in inexpensive and unsophisticated systems, are relatively stable and readily amenable to genetic manipulation. For these reasons, the equine encephalomyelitis viruses are considered classic biological warfare threats. No specific therapy exists for infections caused by these viruses. A live attenuated vaccine for VEE (TC-83) and inactivated vaccines for VEE, EEE, and WEE have been developed and are used under the Food and Drug Administration's investigational new drug status. Although these vaccines are useful in protecting at-risk individuals, they have certain disadvantages, and improved vaccines are under development.

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