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Animal models of highly pathogenic RNA viral infections: Encephalitis viruses

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Abstract

The highly pathogenic RNA viruses that cause encephalitis include a significant number of emerging or re-emerging viruses that are also considered potential bioweapons. Many of these viruses, including members of the family *Flaviviridae*, the genus *Alphavirus* in the family *Togaviridae*, and the genus *Henipavirus* in the family *Paramyxoviridae*, circulate widely in their endemic areas, where they are transmitted by mosquitoes or ticks. They use a variety of vertebrate hosts, ranging from birds to bats, in their natural life cycle. As was discovered in the United States, the introduction of a mosquito-borne encephalitis virus such as West Nile virus can cause significant health and societal concerns. There are no effective therapeutics for treating diseases caused by any of these viruses and there is limited, if any, vaccine availability for most. In this review we provide a brief summary of the current status of animal models used to study highly pathogenic encephalitic RNA viruses for the development of antiviral therapeutics and vaccines.

Keywords: Flavivirus; Alphavirus; Henipavirus; Encephalitis; Small animal model

1. Introduction

The objective of this review is to provide a brief guide to animal models used for the study of highly pathogenic RNA viruses that cause encephalitis (HPRVE). An accompanying review article in this issue discusses RNA viruses associated with viral hemorrhagic fever (Gowen and Holbrook, 2008). The HPRVE are found primarily in three families: *Flaviviridae*, *Togaviridae* and *Paramyxoviridae* and single genera within each family: *Flavivirus*, *Alphavirus* and *Henipavirus*, respectively. In humans, these diseases usually take the form of meningioencephalitis, with limited involvement of the limbs. However, some diseases, such as Japanese encephalitis and Russian spring–summer encephalitis viruses, can cause a polio-like illness with significant peripheral neurologic involvement.

All of the viruses discussed in this review are zoonotic agents, for which human infection is incidental and is a dead-end in the virus life cycle. All of them, with the possible exception of West Nile virus, are considered potential biothreat agents. The majority are transmitted by arthropods with the viruses transmitted to humans in mosquito or tick saliva. The amount of virus required to infect humans is unknown. The route of progression through the host is not clearly established, but it appears that the virus progresses first from the site of the bite to draining lymph nodes, where it replicates and is amplified. The virus then enters the circulation and crosses the blood–brain barrier (BBB) and enters the central nervous system (CNS) through unknown mechanisms. Several hypotheses have been offered for mechanisms of CNS penetration. These include virus penetration as a result of inflammation and damage to vascular integrity (Lossinsky and Shivers, 2004), entry through the olfactory bulb (Cook and Griffin, 2003), toll-like receptor mediated entry (Wang et al., 2004) and transcytosis across vascular endothelial cells (Lossinsky and Shivers, 2004).

The henipaviruses, which are also discussed in this review, are not transmitted by arthropods, with fruit bats (*Pteropus* spp.) serving as the primary reservoir. With Nipah virus, pigs play an important role in viral transmission to humans and it seems likely that transmission to pigs and humans may occur via feces or urine from bats, with transmission from pigs to humans possibly

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following a similar route (Eaton et al., 2005), but this mechanism is not clearly established.

Viruses that cause encephalitis in humans also frequently cause severe neurologic disease in mice, making the mouse model a fundamental tool for studying viral pathology and for the development of vaccines and antivirals. The US Food and Drug Administration (FDA) has stipulated that vaccines and therapeutics considered for use in humans against some highly pathogenic viruses may bypass human efficacy testing if they show efficacy in animal models that are adequately representative of the human disease. This so-called "Animal Rule" is discussed more extensively elsewhere in this issue (Roberts et al., 2008). The "Animal Rule" provides an important avenue for the development of vaccines and therapeutics that would have been impossible if clinical or field efficacy studies were required, and makes the development and recognition of animal models that mimic human disease vital for effective drug development.

A number of animal models have been tested for the HPRVE ranging from guinea pigs to cats and also non-human primate models such as rhesus and cynomolgus macaques. For the viruses discussed here, challenge by peripheral routes is generally sufficient to cause neurologic disease in the established models. One of the limitations of using animals to model human disease is that in order to effectively test antiviral or vaccine efficacy, the animal model needs to have an easily measurable end-point for efficacy evaluation. This end-point is typically debilitating disease or death and many of the viruses discussed here are uniformly lethal in various animal models if a sufficient virus dose is given. Clearly these viruses are not 100% lethal in humans as many of these viruses cause a large number of subclinical infections for each case of disease.

Unlike hemorrhagic fever viruses, the development of encephalitis and neurologic disease in animal models is generally obvious, with paralysis and seizures being measurable indications of illness. The question that arises is whether the mechanisms (e.g. host immune response) of disease development in the animal model are the same as, or similar to, what occurs in humans. Given that some therapeutics and all vaccines rely on the host immune response, gaining a clear understanding of how viral infection affects the host is a major component of effective drug development that is often overlooked or ignored. A second component of effective drug development for encephalitis viruses is the ability to develop drugs that cross the blood–brain barrier and are able either to limit viral replication in the CNS or to reduce the inflammation stimulated by infection.

There are currently no therapeutic interventions and limited, if any, vaccine availability for the diseases discussed in this article. The development and use of animal models for the study of pathogenesis is vital for the development of effective therapeutics and vaccines. Furthermore, the inability to directly determine antiviral or vaccine efficacy in humans makes the use of animal models a vital part of drug development.

The goal of this review is to provide a brief summary of the available animal models for the HPRVE with selected references describing the use of these systems. More comprehensive reviews containing extensive lists of primary references, such as articles covering arboviral encephalitides by Nalca et al. (2003) or flavivirus mouse and hamster models by Charlier et al. (2006), should be consulted for more complete information.

2. Flaviviral encephalitis models

The flaviviruses associated with severe encephalitis are found worldwide and are transmitted by either mosquitoes or ticks. The mosquito-borne encephalitic flaviviruses are all within the Japanese encephalitis serocomplex and are transmitted by *Culex* spp. mosquitoes. The majority of tick-borne flaviviruses cause encephalitis and are found principally in Eastern Europe and Asia. These viruses are transmitted by *Ixodes* spp. of ticks.

2.1. Japanese encephalitis serocomplex viruses

The Japanese encephalitis (JE) serocomplex of viruses are mosquito-borne agents with a wide range of vertebrate hosts. West Nile virus (WNV), for example uses birds as part of its natural cycle, while pigs are a significant component of JE virus (JEV) transmission cycles. Viruses in this serocomplex are found throughout the world with WNV endemic in Africa, Europe and the Americas, JEV in eastern Asia, St. Louis encephalitis virus (SLEV) in the Americas and Murray Valley encephalitis virus (MVEV) in Australia. The development of antiviral therapeutics against JE is discussed elsewhere in this issue (Gould et al., 2008).

The vast majority of pathogenic flaviviruses are associated with neurologic disease (Table 1). These agents cause encephalitis in mice, typically with loss of balance, hind-limb paralysis and total paralysis, with few clear distinctions between the viruses. Japanese encephalitis serocomplex viruses, including JEV, WNV and SLEV are typically lethal to juvenile mice regardless of the route of inoculation and these animals develop severe neurologic disease (Nalca et al., 2003). However, there is some variation among strains of viruses following peripheral inoculation, as some strains and virus genotypes are completely attenuated (Beasley et al., 2002), while others are lethal (e.g. New York99 strains of WNV). Suckling mice are also susceptible to infection by JEV serocomplex viruses, again with some variation between strains, but do not represent an optimal model, as they lack a competent immune system.

Hamsters have also been used as a model for JEV and WNV. Intracerebral and intranasal inoculation of hamsters with JEV is lethal while peripheral inoculation typically caused a non-lethal infection (Burke and Monath, 2001). WNV infection in hamsters also identified variability between strains (Beasley et al., 2002), but those animals that did become ill developed a lethal neurologic disease (Xiao et al., 2001). Animals that survive the acute illness can develop a persistent infection that includes shedding of the virus in the urine (Tonry et al., 2005).

Non-human primates have also been used for studies of JEV and WNV pathogenesis. JEV infection of both rhesus and cynomolgus macaques causes lethal disease when the virus is introduced intranasally, but not following peripheral inoculation (Burke and Monath, 2001). Intracerebral inoculation of WNV causes disease ranging from asymptomatic infection to fatal encephalitis, depending on the strain (Pogodina et al., 1983).

Table 1
Principal animal models for the study of flaviviral encephalitis

Virus	Vector	Geographic location	Animal model	Selected references for the animal model	Selected references for drug and vaccine development
Japanese encephalitis	Mosquito	Eastern Asia	Mouse	German et al. (2006)	Wu et al. (2003), Murakami et al. (2005), Lee et al. (2006)
Japanese encephalitis	Mosquito	Eastern Asia	Hamster	Burke and Monath (2001)	
Japanese encephalitis	Mosquito	Eastern Asia	Non-human primate	Myint et al. (1999), Raengsakulrach et al. (1999)	Tanabayashi et al. (2003), Dean et al. (2005)
West Nile	Mosquito	Africa, Europe, Americas	Mouse	Beasley et al. (2002), Nalca et al. (2003)	Bai et al. (2007), Chu et al. (2007), McDonald et al. (2007)
West Nile	Mosquito	Africa, Europe, Americas	Hamster	Xiao et al. (2001), Tesh et al. (2005), Tonry et al. (2005)	Tesh et al. (2002), Morrey et al. (2004), Morrey et al. (2006)
West Nile	Mosquito	Africa, Europe, Americas	Non-human primate	Pogodina et al. (1983)	Pletnev et al. (2006), Wolf et al. (2006)
St. Louis encephalitis	Mosquito	Americas	Mouse	Murphy et al. (1968), Monath et al. (1980)	Phillpotts et al. (1996), Phillpotts et al. (2003)
St. Louis encephalitis	Mosquito	Americas	Guinea pig	Nalca et al. (2003)	
St. Louis encephalitis	Mosquito	Americas	Hamster	Monath et al. (1983), Siirin et al. (2007)	
St. Louis encephalitis	Mosquito	Americas	Non-human primate	Monath et al. (1980)	
Tick-borne encephalitis	Tick	Europe, Asia	Mouse	Silber and Soloviev (1946), Pogodina (1960b), Popovici et al. (1993)	Danilov et al. (1996), Phillpotts et al. (2003), Labuda et al. (2006)
Tick-borne encephalitis	Tick	Europe, Asia	Non-human primate	Zlontnik et al. (1976), Pogodina et al. (1981a), Pogodina et al. (1981b), Pogodina et al. (1981c), Frolova and Pogodina (1984), Frolova et al. (1985), Kenyon et al. (1992)	Pletnev et al. (2001), Lai and Monath (2003), Rumyantsev et al. (2006)
Powassan	Tick	Asia, North America	Mouse	Holbrook et al. (2005)	Price and Thind (1973)
Powassan	Tick	Asia, North America	Non-human primate	Frolova et al. (1985)	Price and Thind (1973)

There is also evidence of viral persistence in non-human primates regardless of the route of infection where the virus is found in a range of tissues (Pogodina et al., 1983).

St. Louis encephalitis virus (SLEV) is also lethal to mice regardless of the route of inoculation, but there is some variability among strains (Monath et al., 1980). Several other animal models have also been used to study SLEV pathogenesis including rats, guinea pigs, rabbits and non-human primates (Nalca et al., 2003). The related MVEV causes lethal disease in juvenile (<3-week-old) mice by all routes of inoculation, but is less pathogenic in older mice following peripheral inoculation.

2.2. *Tick-borne encephalitis serocomplex viruses*

Tick-borne flaviviruses that cause human disease are found primarily in Eastern Europe and Asia, with only Powassan virus (POWV) found outside of this region. The majority cause lethal encephalitis in mice when inoculated by all routes. The exceptions are Langat virus (LGTV), which is generally apathogenic in adult mice (>4-week-old), and the Omsk hemorrhagic fever virus. Kyasanur Forest disease virus also tends to cause encephalitis in mice despite its association with hemorrhagic fever in humans. POWV, which is found in North America and parts of Russia (Burke and Monath, 2001), causes lethal encephalitis in mice (Holbrook et al., 2005) and experimentally infected non-human primates following intracerebral inoculation (Frolova et al., 1985).

The tick-borne encephalitis viruses (TBEV) including Western subtype TBEV (otherwise known as Central European encephalitis virus) and Far-eastern subtype TBEV (also known as Russian spring–summer encephalitis virus) cause lethal encephalitis in mice regardless of age or route of inoculation, including oral inoculation (Pogodina, 1960a). Many other experimental animals are susceptible to TBEV infection, including guinea pigs, hamsters, rats and non-human primates. There is some degree of strain-specific disease severity, and there are also reports of viral persistence and chronic infection in non-human primates (Smorodintsev, 1958; Nalca et al., 2003), a phenomenon that has also been reported in humans (Gritsun et al., 2003). The far-eastern subtype of TBEV causes much more severe disease in humans than the Western subtype, but these differences are not clearly translated into animal models.

3. Togaviral encephalitis models

The alphaviruses are the primary genus of the *Togaviridae* associated with encephalitic disease in humans. The vast majority are transmitted by mosquitoes. They consist of the Old World alphaviruses such as Ross River virus (RRV), Sindbis (SINV), chikungunya (CHIKV) and Semliki Forest viruses (SFV) which are found primarily in Africa, and the New World

Table 2
Principal animal models for the study of alphaviral encephalitis

Virus	Vector	Geographic location	Animal model	Selected references for the animal model	Selected references for drug and vaccine development
Venezuelan equine encephalitis	Mosquito	Central-South America, Mexico	Mouse	Calisher and Maness (1974), Jackson et al. (1991), Steele et al. (1998), Charles et al. (2001), Ludwig et al. (2001)	Phillpotts et al. (2002), Phillpotts et al. (2003), Paessler et al. (2006), Phillpotts (2006), Steele et al. (2006)
Venezuelan equine encephalitis	Mosquito	Central-South America, Mexico	Hamster	Jackson et al. (1991)	Turell et al. (1999), Pratt et al. (2003)
Venezuelan equine encephalitis	Mosquito	Central-South America, Mexico	Guinea pig	Calisher and Maness (1974), Scherer and Chin (1977), Scherer et al. (1979)	Canonico et al. (1982), Rao et al. (2006)
Venezuelan equine encephalitis	Mosquito	Central-South America, Mexico	Non-human primate	Monath et al. (1974), Jahrling et al. (1977a), Jahrling et al. (1977b), Pratt et al. (1998)	Stephen et al. (1979), Pratt et al. (2003), Reed et al. (2005b), Rao et al. (2006)
Western equine encephalitis	Mosquito	Americas	Mouse	Monath et al. (1978), Bianchi et al. (1993), Bianchi et al. (1997), Nagata et al. (2006)	Schoepp et al. (2002), Nagata et al. (2005), Barabe et al. (2007), Wu et al. (2007)
Western equine encephalitis	Mosquito	Americas	Hamster	Zlotnik et al. (1972)	Cole and McKinney (1969), Cole et al. (1972), Julander et al. (2007)
Western equine encephalitis	Mosquito	Americas	Guinea pig	Bianchi et al. (1997)	
Western equine encephalitis	Mosquito	Americas	Non-human primate	London et al. (1982), Reed et al. (2005a)	Moreland et al. (1979), Reed et al. (2005a)
Eastern equine encephalitis	Mosquito	Americas	Mouse	Murphy and Whitfield (1970), Brown et al. (1975), Vogel et al. (2005)	Brown and Officer (1975), Schoepp et al. (2002)
Eastern equine encephalitis	Mosquito	Americas	Hamster	Paessler et al. (2004)	Cole and McKinney (1969)
Eastern equine encephalitis	Mosquito	Americas	Guinea pig	Sorrentino et al. (1968), Nalca et al. (2003)	
Eastern equine encephalitis	Mosquito	Americas	Non-human primate	Wyckoff and Tesar (1939)	

alphaviruses, which include western, eastern and Venezuelan equine encephalitis viruses. The Old World viruses SINV and CHIKV cause arthritic disease, which in the case of CHIKV can be very severe and occasionally fatal. SFV rarely causes disease in humans (Willems et al., 1979; Mathiot et al., 1990) although not much is known about human exposure or seroprevalence in endemic areas. The New World viruses, which cause severe encephalitis in humans that is frequently fatal, are a topic of this review (Table 2).

3.1. Venezuelan equine encephalitis virus

Venezuelan equine encephalitis virus (VEEV) causes severe encephalitis in humans and horses. During epidemic outbreaks, the principal mosquito vectors include *Aedes* spp. and *Psorophora* spp. The virus is also lethal in a number of laboratory models, including mice, hamsters, guinea pigs and non-human primates (reviewed in (Nalca et al., 2003)). In guinea pigs and hamsters, VEEV causes an acute febrile disease without indications of neurologic illness, while in mice, VEEV causes a severe neurotropic disease similar to that seen in horses and humans (Berge, 1958; Gleiser et al., 1962; Jackson et al., 1991; MacDonald and Johnston, 2000). In mice, VEEV initially targets lymphoid organs, as it does in all model systems, then enters the brain via the olfactory system (Berge, 1958; Gleiser et al., 1962; Gorelkin and Jahrling, 1975; Nalca et al., 2003). In non-human primates, VEEV infection is not characterized by neurologic disease, despite evidence of CNS invasion, but rather by an acute biphasic disease that targets lymphoid organs (Berge, 1958; Gleiser et al., 1962; Monath et al., 1974; Nalca et al., 2003).

3.2. Western equine encephalitis virus

Western equine encephalitis virus (WEEV) is found in North and South America. There have been relatively few cases of WEE in the United States, and it is thought that the virus circulates primarily in South America. Most human cases are associated with epizootics in horses. The virus can infect a number of birds and some small mammals including squirrels, bats, rabbits and hares (Hardy, 1987; Calisher, 1994; Ubico and McLean, 1995; Nalca et al., 2003). In the laboratory, WEEV has been shown to infect mice, hamsters and guinea pigs (Aguilar, 1970; Liu et al., 1970; Monath et al., 1978). It causes a rapid acute disease in suckling mice, with the virus showing a specific tropism for cardiac tissue (Aguilar, 1970). In adult mice, WEEV causes a more protracted illness, with afflicted animals developing encephalitis about 10 days post-infection, with variations in the disease course depending upon the route of inoculation and dose of virus. The induced pathology is seen in both visceral and neural tissues (Monath et al., 1978). WEEV infection of hamsters is lethal regardless of the route of infection, with the animals developing a severe neurologic disease with brain hemorrhage common following both intracerebral and peripheral

inoculation (Zlotnik et al., 1972). In guinea pigs, WEEV also causes severe disease, with death occurring in less than 10 days (Bianchi et al., 1997; Nalca et al., 2003). WEEV also causes lethal encephalitis in non-human primates (Reed et al., 2005a).

3.3. Eastern equine encephalitis virus

Eastern equine encephalitis virus (EEEV) is associated with outbreaks of severe encephalitis in horses throughout the eastern United States, into Texas and Central and South America. Human disease usually occurs in association with equine outbreaks, but isolated cases occur occasionally in endemic regions. In the United States, human infection is frequently associated with neurologic disease, while in South America the disease is typically mild or subclinical (Causey et al., 1961) although, based on the prevalence of virus in mosquitoes and small mammals (Bigler et al., 1976; Pagac et al., 1992; Day et al., 1996; Wozniak et al., 2001; Loftin et al., 2006), many cases in the United States may also be inapparent. EEEV is transmitted by culicine mosquitoes and is thought to be maintained in birds, which remain asymptomatic despite having a high viremia.

EEEV causes disease in a number of laboratory animals, including mice, guinea pigs, hamsters, non-human primates and some bird species, including chickens (Wyckoff and Tesar, 1939; Morgan, 1941; Tyzzer and Sellards, 1941; Luginbuhl et al., 1958; Liu et al., 1970; Murphy and Whitfield, 1970; Dremov et al., 1978; Scott and Weaver, 1989; Griffin, 2001). It causes lethal encephalitis in suckling and adult mice following intracerebral inoculation and a pantropic infection with encephalitis following peripheral inoculation (Morgan, 1941; Liu et al., 1970; Murphy and Whitfield, 1970). EEEV also causes encephalitis in hamsters and guinea pigs (Dremov et al., 1978). Infection of non-human primates by intracerebral inoculation results in lethal illness, but peripheral inoculation causes subclinical infection (Wyckoff and Tesar, 1939). Intranasal or aerosol infection results in uniformly lethal neurologic disease in cynomologous macaques (Reed et al., 2007).

4. Paramyxoviral encephalitis models

The paramyxoviruses include a wide variety of animal and human pathogens. The emerging Nipah (NIPV) and Hendra (HENV) viruses are unusual agents with the ability to cause severe encephalitis in humans following close contact with infected pigs and horses, respectively (Eaton, 2001). Although these two viruses are genetically similar, they are sufficiently different from other paramyxoviruses to warrant classification into the new genus *Henipavirus*. The broad host range and high mortality rates associated with these highly virulent viruses has led to the requirement of maximum (BSL-4) containment for their study, which has greatly restricted access by the scientific community.

4.1. Nipah virus

In pigs, NIPV infections are generally associated with respiratory complications, whereas severe infections of humans predominantly present as acute encephalitis, with less frequent occurrence of respiratory disease. Remarkably, severe encephalitis has been documented several years after asymptomatic infection, and relapse following encephalitis has also been reported (Eaton et al., 2006). This suggests that NIPV persists for long periods, and that some form of antiviral intervention may be required to eradicate the virus.

Two animal models have been developed to study NIPV infection (Table 3). The hamster model appears to reproduce the disease seen in humans, as animals die of acute encephalitis following peritoneal or intranasal challenge (Wong et al., 2003). To date, several studies employing the hamster model have been conducted to assess the efficacy of nucleoside analogues, passive immunization and vaccination strategies (Guillaume et al., 2004; Georges-Courbot et al., 2006). The other NIPV infection model is based on subcutaneous challenge of cats (Mungall et al., 2006), which are naturally susceptible to the virus (Hooper et al., 2001). Despite evident similarities in viral tropism and pathology (Middleton et al., 2002), the feline model does not appear to recapitulate the encephalitis principally seen in human cases.

4.2. Hendra virus

Similar to the transmission of NIPV from infected pigs to humans, HENV infection is a zoonosis resulting from exposure to infected horses. As with NIPV, symptomatic human disease predominantly manifests as a severe acute encephalitis, with less frequent respiratory illness and evidence of recurrence (Eaton et al., 2006). HENV cases are less common, so the pathogenesis in humans and susceptible animal species has not been characterized to the same degree as for NIPV. A guinea pig model appears to resemble human disease, with virus detectable and recoverable from encephalitic brains (Williamson et al., 2001). The model is yet to be used for the evaluation of countermeasures.

Table 3
Principal animal models for the study of paramyxoviral (*Henipavirus*) encephalitis

Virus	Natural host	Geographic location	Animal model	Selected references for the animal model	Selected references for drug and vaccine development
Nipah	Bats (Flying Fox-Genus *Pteropus*)	Bangladesh, Malaysia, Singapore	Hamster	Wong et al. (2001)	Guillaume et al. (2004), Georges-Courbot et al. (2006)
Nipah	Bats (Flying Fox-Genus *Pteropus*)	Bangladesh, Malaysia, Singapore	Cat	Middleton et al. (2002), Mungall et al. (2006)	Mungall et al. (2006)
Hendra	Bats (Flying Fox-Genus *Pteropus*)	Northeastern Australia	Guinea pig	Williamson et al. (2001)	

Further characterization of HENV infection in other guinea pig strains may be beneficial in identifying the most suitable system in which lethal encephalitis can consistently be produced experimentally.

5. Concluding remarks and future directions

The highly pathogenic RNA viruses that cause encephalitis are largely transmitted by arthropod vectors, primarily mosquitoes. The introduction of a new pathogen into a naïve population or an outbreak in an endemic area can therefore spread easily and rapidly beyond an index case. This scenario has played out quite obviously in recent outbreaks of West Nile (Sejvar, 2006) and chikungunya (Powers and Logue, 2007) viruses in North America and East Africa, respectively. Several of these viruses, most prominently VEEV, are considered potential bioweapons and were developed as such prior to the worldwide treaty to ban development and stockpiling of biological weapons signed in 1972.

As there are no viable specific treatments available for any of these infections, it is imperative that extensive studies be performed that address the development of antiviral therapeutics. A significant issue for the development of effective therapies for the treatment of viral encephalitis is the ability of the therapeutic agent to cross the BBB and limit virus replication or the host immune response. To date there are very few drugs that are capable of crossing the BBB, none of which are effective against the viruses discussed here, but there is clearly a significant effort to identify mechanisms of transport and novel therapeutic strategies for treatment of neurologic disease (Strazielle and Ghersi-Egea, 2005; Boado, 2007; de Boer and Gaillard, 2007; Jones and Shusta, 2007; Kumar et al., 2007; Pardridge, 2007).

There is also a significant need for vaccines for most of these viruses, as JEV is the only agent discussed in this review for which there is an FDA-approved vaccine. An IND vaccine for VEE (TC-83) is available in the United States, but only from the US Army, and at great expense. There are also IND vaccines for EEEV and WEEV, but these are typically given only to laboratory workers, and are difficult to obtain and very expensive. A vaccine for chikungunya is in clinical trials (Edelman et al., 2000) and two vaccines for TBE are licensed in Europe, but not the United States (Barrett et al., 2003). The development and use of animal models, especially small animals, is vital for an increased understanding of viral pathogenesis and development of therapeutics and vaccines.

A wide variety of models have been tested for various encephalitis viruses, but many differ significantly from the human disease. Based on serology studies, these viruses cause symptomatic illness in only a small percentage of infected humans, and an even smaller percentage develop encephalitis. As mentioned earlier, effective animal models require a measurable end-point, which is usually death, for measurement of drug efficacy. However, using a model in which only a small fraction of animals develop significant disease is an inefficient use of animals and would likely not be acceptable to most animal use committees or funding agencies. For most of the diseases discussed here, the mouse is the model of choice, as these animals are inexpensive, easy to house and have an extensive array of reagents available to study cellular and humoral responses. Larger animals such as hamsters and guinea pigs are also used, but both models suffer from a lack of reagents to assess immune responses. Perhaps, given an increased use of these systems, more reagents will become commercially available.

For many of the viruses discussed here, non-human primates are optimal for comparison with human disease. However, the use of these animals for research can be prohibitively expensive and logistically complicated. Additionally, given that the majority of the viruses discussed here are BSL-3 or BSL-4 agents, work with non-human primates is severely restricted by the availability of adequate containment facilities.

The FDA has recognized that drug and vaccine efficacy trials for highly pathogenic agents often are not practical in humans, and has allowed for studies in animals to be a reasonable substitute (Roberts et al., 2008). This decision has made the development and complete characterization of animal models a more vital component of viral research and drug development. While simple experiments examining the protective capacity of drugs or vaccines (“feet up-feet down”) are indicative of drug efficacy, in order for a model to be truly effective, host responses to infection and therapy must be determined. Without a thorough understanding of the model itself, its effectiveness for testing drugs and vaccines and their correlation to human disease cannot be clearly determined. As such, much work remains to fully evaluate these animal models and to develop reagents that let us make effective use of them.

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