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Review

Rodent models for the study of therapy against flavivirus infections

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Abstract

Flaviviruses cause a variety of diseases including (meningo)encephalitis and hemorrhagic fevers. There is no specific antiviral therapy available for the treatment of infections with flaviviruses and such therapy should be urgently developed. Small animal models that are reminiscent of the disease in man will be instrumental to identify therapeutic strategies against flavivirus infections. Here we review models in mice and hamsters that may be used to assess the efficacy of novel antiviral strategies against flavivirus infections.

Keywords: Flavivirus; Animal model; Mouse; Hamster

1. Introduction

Flaviviruses are enveloped, positive single-stranded RNA viruses that belong, together with the Hepaci- and Pestiviruses, to the family of the *Flaviviridae*. The genus *Flavivirus* contains (i) viruses that are transmitted by mosquitoes or ticks (arthropod-borne) and (ii) viruses with no known vector (NKV) (Chambers et al., 1990) (Fig. 1). Currently, more than 70 flaviviruses have been reported. All flaviviruses of human importance belong to the arthropod-borne group. The NKV-group holds a few viruses which have been isolated from mice or bats and for which no arthropod-borne or natural route of transmission has (yet) been demonstrated (Kuno et al., 1998).

One of the most important flaviviruses causing disease in man is dengue virus (DENV). It is estimated that there are worldwide annually as many as 50–100 million cases of dengue fever and several hundred thousand cases of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), the latter with an overall case fatality rate of about 5% (Gubler, 1997; Thomas et al., 2003).

Yellow fever virus (YFV) is, despite the availability of a highly efficacious vaccine, still a leading cause of hemorrhagic fever worldwide. The World Health Organization has estimated that there are annually 200,000 cases of YF, including 30,000 deaths, of which over 90% occur in Africa (http://www.who.int/inf-fs/en/fact100.html).

Japanese encephalitis (JE), a mosquito-borne arboviral infection, is the leading cause of viral encephalitis in Asia (Hennessy et al., 1996). Approximately 50,000 sporadic and epidemic cases of JE have been reported annually. The infection results in high mortality (30%), and about half of the survivors develop long-lasting neurological sequelae (Kalita and Misra, 1998; Misra et al., 1998).

Other important flaviviruses that cause encephalitis are also responsible for high mortality rates or neurological sequelae. Tick-borne encephalitis virus (TBEV) is believed to cause annually at least 11,000 human cases of encephalitis in Russia and about 3000 cases in the rest of Europe (http://www.who.int/). Related viruses within the same group are Louping ill virus (LIV), Langat virus (LGTV) and Powassan virus (POWV). LIV is primarily known as a disease of sheep, but has also been shown to infect, and cause disease in deer, cattle, goats, red grouse and occasionally in man (Davidson et al., 1991; Monath and Heinz, 1996). LGTV and POWV also cause human encephalitis but, as for LIV, rarely on an epidemic scale (Burke and Monath, 2001). Three other viruses within the same group, Omsk hemorrhagic fever virus (OHFV), Kyasanur Forest disease virus (KFDV) and Alkhurma virus (ALKV), are closely related

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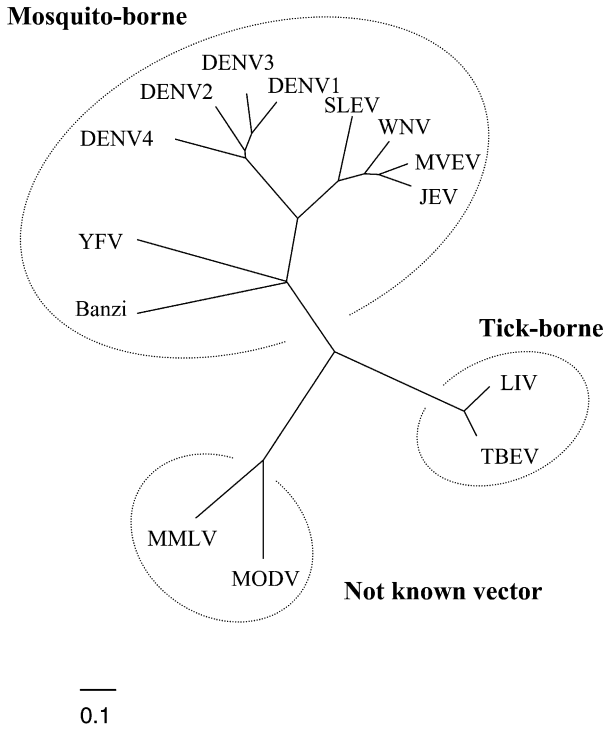

Fig. 1. Phylogenetic tree including the flaviviruses discussed in this review.

to the TBE complex viruses and cause fatal hemorrhagic fevers rather than encephalitis (Gritsun et al., 2003). Murray Valley encephalitis virus (MVEV), a virus belonging to the JE antigenic complex, causes encephalitis in man. Although the last large epidemic caused by MVEV occurred in 1974, new cases of MVEV infection are reported regularly, especially in Western Australia (McCormack and Allworth, 2002). In 1996 an outbreak of West Nile (WN) encephalitis with 373 cases and 17 deaths was reported in Romania (Han et al., 1999; Tsai et al., 1998). In 1999, the disease appeared for the first time in the northeastern United States and has continued to spread across the United States and Canada. In 2003, 9388 human cases of WN fever, WN (meningo)encephalitis and 246 deaths were reported in the USA (http://www.cdc.gov/ncidod/dvbid/westnile/). Outbreaks of WN encephalitis occurred in recent years also in Southern Russia and Israel (Lvov et al., 2000; Siegel-Itzkovich, 2000). St. Louis encephalitis virus (SLEV) is endemic in the western United States and is responsible for severe neurologic disease (Kramer et al., 1997).

Despite the clinical impact of infections with flaviviruses, there is as yet no effective therapy. Treatment is supportive and consists of good medical management and nursing care. Here, we review various models for flavivirus infections in small laboratory animals. Some models may be readily amenable for the evaluation of antiviral strategies.

2. Mouse and hamster models for flavivirus infections

Most flaviviruses that cause infection in man, require special research facilities, i.e., bio-safety level 3 (BSL-3) laboratories for manipulation of, e.g., YFV, WNV, JEV, LIV, MVEV, POWV, and even BSL-4 containment facilities for tick-borne encephalitis viruses such as Russian spring–summer encephalitis virus (RSSEV) and Omsk hemorrhagic fever virus (OHFV) (http://www.cdc.gov/od/ohs/biosfty/bmbl/sect7f.htm). Ideally, a virus used in an animal model for the evaluation of novel antiviral strategies should (i) be a BSL-2 pathogen, (ii) cause morbidity and mortality in small (adult) laboratory animals following systemic inoculation, (iii) not be pathogenic to man, and (iv) closely mimic disease in man. In case that a particular compound would prove active against (a selection of) flaviviruses in cell culture, the prophylactic or therapeutic value of such compound(s) should be assessed in an experimental rodent model. If good activity is observed in a small animal model, further preclinical efficacy studies may be carried out in monkey models.

2.1. Flaviviruses primarily associated with fever, arthralgia and rash

2.1.1. Dengue virus

The mouse is not the natural host of dengue virus. So far there are only three known natural hosts for DENV infections: mosquitoes, humans, and lower primates (reviewed by Gubler, 1994). In baby mice, unadapted DENV strains usually produce subclinical infections and, sporadically, illnesses leading to paralysis and death. Baby mice that have been challenged with mouse-brain adapted strains develop disease (Meiklejohn et al., 1952). Adult mice that have been inoculated with unadapted DENV strains produce no symptoms. Challenge with highly adapted DENV strains sometimes causes encephalitis in laboratory-bred mice [e.g., NMRI (nu/nu), BL6 (nu/nu)] (Boonpucknavig et al., 1981; Raut et al., 1996).

Severe combined immune deficiency (SCID) mice reconstituted with human peripheral blood lymphocytes (hu-PBL-SCID mice) and infected subsequently by the intraperitoneal route with DENV type 1, developed viremia, although virus production was highly variable (Wu et al., 1995). DENV type 4 replicates in SCID mice that have been transplanted with HuH-7 cells (human liver cells) (Blaney et al., 2002). SCID mice that had been engrafted with human K562 cells (an erythroleukemia cell line) and that were subsequently inoculated with DENV type 2 developed paralysis and died. A high titer of DENV type 2 was found in the brain, which correlated well with the progression of encephalopathy (Lin et al., 1998). Mouse-adapted DENV type 2 replicates in intraperitoneally infected AG129 mice (which lack IFN-α/β and IFN-γ receptor genes). Infected animals developed neurological abnormalities, including hindleg paralysis and blindness at 7 days, and died within two weeks following infection (Johnson

and Roehrig, 1999). An et al. (1999) transplanted SCID mice with a human hepatocarcinoma cell line (HepG2) and inoculated the animals intraperitoneally with DENV type 2 about 7–8 weeks after transplantation. In the early stage post-infection, virus was detected in the liver and in serum of the HepG2-engrafted mice. At later time-points, virus was also detected in the brain, and this was corroborated by the fact that the animals developed paralysis. The mice also presented with thrombocytopenia and a prolonged partial thromboplastin time, which is a characteristic for DHF. Also an increase in hematocrit, blood urea nitrogen, and tumor necrosis factor alpha (TNF-α) were observed in paralyzed mice. TNF-α might be involved in the etiology of shock in DHF/DSS. Moreover, mild hemorrhage in the liver and tarry stool in the small intestine were noted in some mice (An et al., 1999).

Huang and colleagues developed another infectious model that mimics to some extent DHF/DSS (Huang et al., 2000). A/J strain mice infected intravenously with DENV type 2 (PL046), developed manifest thrombocytopenia and produced anti-platelet antibody. Anti-platelet antibodies lyse platelets in the presence of complement and interfere with thrombin-induced platelet aggregation (Huang et al., 2000). Immune-mediated clearance of platelets has been shown to be involved in the pathogenesis of thrombocytopenia in DHF/DSS (Wang et al., 1995).

As mentioned above, young mice that are inoculated with unadapted DENV strains produce normally no symptoms. Yet a lethal mouse model for DENV type 2 infection was recently reported in which infected mice developed several clinical, histopathological, virological and hematological similarities to human DENV type 2 infection. Young BALB/c mice (haplotype H-2d) infected with DENV type 2 (strain 23085), developed anemia, thrombocytopenia, pre-terminal paralysis and shock. Levels of TNF-α abruptly and steeply increased 24 h before death (mean at day 6). Serum levels of IL-1β, IL-6, IL-10, IL-1 receptor antagonist and soluble TNF receptor I continuously increased with time after infection. A 100% mortality rate was noted in the infected animals; treatment with anti-TNF-α serum reduced mortality from 100% to 40% (Atrasheuskaya et al., 2003). The different mouse models for DENV infections described here are summarized in Table 1.

Table 1
Mouse models for dengue virus infections

Mice	Virus serotype	Reference
SCID transplanted with human peripheral blood lymphocytes	DENV1	Wu et al. (1995)
SCID transplanted with human liver cells	DENV4	Blaney et al. (2002)
SCID transplanted with human K562 cells	DENV2	Lin et al. (1998)
SCID transplanted with a human hepatocarcinoma cell line	DENV2	An et al. (1999)
AG129 /(IFN-α/β and IFN-γ receptor genes knockout)	DENV2	Johnson and Roehrig, (1999)
A/J	DENV2	Huang et al. (2000)
BALB/c	DENV2	Atrasheuskaya et al. (2003)

Besides rodents, monkeys (such as *Macaca fascicularis*, white-handed gibbon) have been used to study DENV infections (Angsubhakorn et al., 1986; Halstead et al., 1973a,b; Whitehead et al., 1970; Malinoski et al., 1990)

2.1.2. West Nile virus

2.1.2.1. Mouse model. In man, WNV infections are often mild and characterized by a self-limiting acute febrile illness accompanied by headaches, myalgia, polyarthropathy, rash, and lymphadenopathy (Goldblum et al., 1954; Tsai et al., 1998; Asnis et al., 2000; Hubalek and Halouzka, 1999). Rarely, acute hepatitis and pancreatitis have been reported (Sampson and Armbrustmacher, 2001; Perelman and Stern, 1974; Sampson et al., 2000; Hubalek and Halouzka, 1999). If the virus crosses the blood–brain barrier, it may cause life-threatening (meningo)encephalitis (Sampson and Armbrustmacher, 2001).

Ben Nathan et al. (2003) have employed a mouse-neuroadapted WNV. The original strain of this virus was isolated in 1952 from the blood of a patient during a febrile phase of the disease (Goldblum et al., 1954). The virus was passaged several times in suckling mice brain and Vero cells. Mortality rates in BALB/c mouse that had been infected intraperitoneally with this mouse-neuroadapted WNV was 100% (Ben Nathan et al., 2003).

The WNV strains of Middle Eastern or African origin proved highly lethal, although the viremia was minimal, to adult Institute of Cancer Research (ICR) mice following intraperitoneal inoculation (Nathanson, 1980; Weiner et al., 1970; Eldadah et al., 1967; Eldadah and Nathanson, 1967; Lustig et al., 2000).

The New York isolate is highly virulent and neuroinvasive in 3- to 4-week-old female NIH Swiss outbred mice following intraperitoneal inoculation (Beasley et al., 2002; Hall et al., 2003).

Strain IS-98-ST1, isolated from a stork in Israel in 1998, and closely related to strain Isr98/NY99 (Deubel et al., 2001; Malkinson et al., 2002), has the capacity to kill adult mice (e.g., BALB/c, ICR) following peripheral inoculation (Mashimo et al., 2002; Xiao et al., 2001a). In the brain, perivascular cuffing (with macrophages and lymphocytes) were found around small vessels. Neuronal degeneration and necrosis, neuronophagia, spongy degeneration, and focal hemorrhages were observed as well. The virus is mainly detected in the hippocampus and cortex areas of the mouse brain (Shrestha et al., 2003; Deubel et al., 2001). In man, monkeys, horses, and birds, preferential sites of virus replication are the thalamus, cerebrum, cerebellum, medulla oblongata and cervical spinal cord (Steele et al., 2000; Pogodina et al., 1983; Sampson et al., 2000).

2.1.2.2. Hamster model. The histopathologic changes reported in the brain and spinal cord of hamsters infected parenterally with WNV are similar to those observed in the WNV-infected adult mice (Weiner et al., 1970; Eldadah and Nathanson, 1967). Following intraperitoneal inoculation with WNV strain 385-99 [a New York isolate which was originally isolated from the liver of a Snowy Owl that died at the Bronx Zoo during the 1999 epizootic in New York City], hamsters developed a viremia of 5–6 days in duration, followed by the development of humoral antibodies (Tesh et al., 2002). The appearance of viral antigen in the brain (i.e., the basal ganglia, brain stem, cerebellar cortex, and gray matter but not within the olfactory bulb) and neuronal degeneration began 6 days after infection and about half of the animals died during the acute fase of the infection (Beasley et al., 2002). WNV was cultured from the brain of convalescent hamsters up to 53 days after initial infection, suggesting that persistent virus infection occurred (Xiao et al., 2001a). The rodent models for WNV infections described here are summarized in Table 2.

As mentioned above, also several bird and monkey species can be infected with WNV and may constitute models for the study of the pathogenesis and therapy of WNV infection (Ratterree et al., 2004; Pogodina et al., 1983; Goverdhan et al., 1992).

2.1.3. Banzi virus

Banzi virus was first isolated from the blood of a febrile child in South Africa in 1956. Analysis of the NS5 gene sequences suggested that the virus is most closely related to the mosquito-borne flaviviruses, in particular yellow fever (Burke and Monath, 2001).

Adult C3H/He mice that have been inoculated intraperitoneally with the Banzi virus, develop a lethal encephalitis with widespread necrosis of brain tissue (Jacoby and Bhatt, 1976). Also intraperitoneal inoculation of Banzi virus in young adult immunocompetent (ICR) mice results in a lethal encephalitis (Singh et al., 1989; Smee et al., 1987; Pinto et al., 1988) (Table 3).

2.2. Flaviviruses primarily associated with encephalitis

2.2.1. Japanese encephalitis virus

In mice, disease outcome following extraneural inoculation with JEV is strongly host factor dependent (Monath, 1986). For example, the age of the animals is particularly important (Grossberg and Scherer, 1966; Macdonald, 1952a,b). Mice up to 3–4 weeks of age are highly susceptible to a low peripheral inoculum of virulent (neuroinvasive) JEV strains. In these mice, the virus is detected in the brain, as well as in various extraneural tissues as well as in the blood (McMinn et al., 1996). In older mice the peripheral injection of viruses that belong to the JEV complex is mostly associated with low or undetectable viremia and often fails to result in encephalitis and death. However, when injected directly into the brain, the viruses grow to high titers and cause a fatal encephalomyelitis (Higgs and Gould, 1991; Licon Luna et al., 2002; Lee and Lobigs, 2002; Huang and Wong, 1963) (Table 3). A low-molecular-weight chemical inducer of interferon, 10-carboxymethyl-9-acridanone (CMA), prevented JEV-induced mortality in weanling mice (Taylor et al., 1980). Oral delivery of the endoplasmic reticulum (ER) α-glucosidase inhibitor *N*-nonyl-deoxynojirimycin (*N*N-DNJ) results in a reduced mortality rate of young ICR mice that had been inoculated with JEV (Wu et al., 2002). Diethyldithiocarbamate (DDTC), an immunomodulator, delayed progression of the disease and prolonged the average survival time of mice infected intracerebrally with a lethal dose of JEV (Saxena et al., 2003).

Other animals that are infectable and that have been reported to constitute models for the study of JEV infections, include piglets which develop encephalitis (Yamada et al., 2004) and rhesus macaques (*Macaca mulatta*) (Myint et al., 1999).

2.2.2. Louping ill virus

LIV produces in mice a fatal encephalitis that is similar to the disease in sheep. Experimentally infected sheep develop prolonged viremia followed by ataxia, paralysis, tremors and death (Doherty and Reid, 1971; Burke and Monath, 2001; Sheahan et al., 2002).

In BALB/c mice (inoculated intraperitoneally or intranasally), infection with the LI/31 strain [prepared in the brain of neonatal BALB/c mice; Fleeton et al., 1999] and MA54 strain (an Irish isolate more closely related than other LIV strains to TBE virus) of LIV was invariably lethal. LI/I (a naturally occurring “antibody escape” mutant) was lethal for the majority of infected animals. Mice infected with these viruses developed a severe and lethal neurological disease (Fleeton et al., 1999, 2000; Sheahan et al., 2002) (Table 3).

Table 2
Mouse and hamster models for West Nile virus infections

Animals	Virus strain	Reference
BALB/C mice	Mouse-neuroadapted strain	Ben Nathan et al. (2003)
ICR mice	Strains of Middle Eastern or African origin	Nathanson (1980), Weiner et al. (1970), Eldadah et al. (1967), Eldadah and Nathanson (1967), Lustig et al. (2000)
Swiss mice	New York isolate	Beasley et al. (2002), Hall et al. (2003)
BALB/c, ICR mice	Strain IS-98-ST1	Mashimo et al. (2002), Xiao et al. (2001a)
Hamsters	Strain 385-99	Tesh et al. (2002), Beasley et al. (2002), Xiao et al. (2001a)

Table 3
Mouse and hamster models for flavivirus infections (other than DEN and WN)

Virus	Animals	Reference
SLEV	Porton outbred TO mice	Phillpotts et al. (1997), Brooks and Phillpotts (1999)
	Syrian hamsters	Monath et al. (1983), Harrison et al. (1982)
TBEV	Outbred white mice	Rajcani and Gresikova (1982)
	BALB/c mice	Kreil and Eibl (1997)
	ICR mice	Chiba et al. (1999)
LIV	BALB/c	Fleeton et al., 1999, 2000, Sheahan et al. (2002)
MODV	SCID mice	Leyssen et al., 2001, 2003
	NMRI mice	Leyssen et al. (2003)
	Syrian hamsters	Leyssen et al. (2001)
MMLV	SCID mice	Charlier et al. (2002b)
Banzi virus	C3H/He mice	Jacoby and Bhatt (1976)
	ICR mice	Singh et al. (1989), Smee et al. (1987), Pinto et al. (1988)
JEV	Various strains including Swiss, C57BL/6, etc	McMinn et al. (1996), Higgs and Gould (1991), Licon Luna et al. (2002), Lee and Lobigs (2002), Huang and Wong (1963)
MVEV	Swiss mice	McMinn et al. (1996)
	ARC/Swiss mice	Hurrelbrink et al. (1999)
YFV	BALB/c TO mice	Barrett and Gould (1986), Burke and Monath (2001)
	CD-1 mice	Chambers and Nickells (2001), Nickells and Chambers (2003)
	Syrian hamsters	Tesh et al. (2001), Xiao et al. (2001b), Arya (2001)

Other experimental models for LIV infections have been described in lambs (Laurenson et al., 2000; Sheahan et al., 2002).

2.2.3. *Murray Valley encephalitis virus*

Inoculation of weanling Swiss mice via the footpad with a highly neuroinvasive field isolate (BH3479) of Murray Valley encephalitis virus (MVEV), results in encephalitis and finally mortality within 10 days post-infection (McMinn et al., 1996). High mortality rates in mice inoculated with this MVEV strain (BH3479) correlated with a marked infiltration of polymorphonuclear and mononuclear leukocytes in the CNS (Matthews et al., 2000). MVEV strain 1-51 proved lethal to young Animal Resources Centre (ARC) Swiss mice that had been inoculated intracranially or intraperitoneally (Hurrelbrink et al., 1999) (Table 3).

2.2.4. *St. Louis encephalitis virus*

2.2.4.1. Mouse model. St. Louis encephalitis virus strains vary considerably in virulence for mice. Virus strains isolated from birds (the usual host for SLEV) are highly virulent, in contrast to strains obtained from rodents and carnivores, which are attenuated. Virulence in intraperitoneally infected mice correlated with high viremia, replication in extraneural tissues and early neuroinvasion (Monath et al., 1980).

Following subcutaneous inoculation with SLEV, mice (Porton outbred TO strain) became ill at 6–8 days after infection. The illness progressed through an increasing paucity of movement, hindlimb paralysis, followed by front limb paralysis, coma and death. SLEV is also infectious (for some strains of mice) by the airborne route. Mortality was 100% when mice were inoculated via inhalation. Animals developed neurological symptoms (including paralysis) and viral replication was detected in the brain (Table 3).

The effect of recombinant human interferon hybrids IFN-α A:D (Roche Laboratories) and IFN-α B:D (Ciba-Geigy) have been explored in these models. Intraperitoneal administration for several days around the time of exposure to the virus or shortly thereafter, resulted in a reduced (30–70%) mortality (Phillpotts et al., 1997; Brooks and Phillpotts, 1999).

2.2.4.2. Hamster model. The mortality rate of adult Syrian hamsters infected intraperitoneally with SLEV (strain TBH-28) was 88%. Virus was detected as of day 4 post-infection (p.i.) in the olfactory neuroepithelium and spread to the olfactory bulbs (day 5 p.i.), and finally to the remainder of the brain (day 6 p.i.) (Monath et al., 1983). In suckling Syrian hamsters that had been infected subcutaneously, replication in extraneural tissues was shown. Significant virus replication was noted in the pancreas, adrenal gland, small intestine, heart, skeletal muscle and kidneys (Harrison et al., 1982) (Table 3).

Besides mice and hamsters, also rhesus monkeys have been experimentally infected with SLEV (Monath et al., 1980).

2.2.5. *Tick-borne encephalitis virus*

Outbred white mice (of various ages) were inoculated subcutaneously with the Skalica strain of TBEV. The appearance and severity of encephalitis and levels of virus in brain tissue decreased with increasing age of the animals (Rajcani and Gresikova, 1982).

TBEV (strain Neudörfl) caused a lethal infection following either subcutaneous or intraperitoneal infection of young adult BALB/c mice. Viremia became first detectable at 24 h post-infection. TBEV was first detected in the brain about 6 days after virus inoculation; titers then increased steadily and remained high until death of the animals. The

mean survival time in this model of TBEV infection was at about day 10 post-infection (Kreil and Eibl, 1997). The Oshima strain, isolated from a sentinel dog in Hokkaido, Japan, proved lethal for 87% of young adult ICR mice that had been inoculated subcutaneously. The Sofjin strain caused a lethal encephalitis in 100% of the animals (Chiba et al., 1999) (Table 3).

Besides mice also dogs have been experimentally infected with TBEV (Weissenbock et al., 1998). *Macaca radiata* (bonnet macaques) have been suggested to mimic Kyasanur Forest disease in man. Infection of these animals could serve as a model for human disease caused by other, related strains of this group of viruses (Pogodina et al., 1981; Kenyon et al., 1992).

2.2.6. Modoc virus and Montana Myotis leukoencephalitis virus

2.2.6.1. Mouse model. The mouse and the bat are the natural hosts of Modoc virus (MODV) and Montana Myotis leukoencephalitis virus (MMLV), respectively; these flaviviruses were first isolated from the white footed deer mouse (*Peromyscus maniculatus*) (MODV) and *Myotis Lucifugus* (MMLV) (Zarnke and Yuill, 1985; Bell and Thomas, 1964).

MODV and MMLV replication in Vero cells appeared to be about as sensitive as YFV and DENV replication to the inhibitory activity of ribavirin and a selection of experimental antiviral molecules. Both viruses are neuroinvasive in immunodeficient mice. Although no major histopathological changes were observed in the brain of SCID mice that had obvious neurological symptoms following MODV (or MMLV) infection, ultrastructural analysis of the neurons revealed a cellular pathology characteristic of flavivirus infection. Viral RNA and/or antigens were detected in the brain (olfactory lobes, cerebral cortex, lymbic structures, midbrain, cerebellum and medulla oblongata and was confined to neurons) and serum of SCID mice infected with MODV or MMLV. The interferon-α/β inducer poly(I).poly(C) and Ampligen® efficiently protected against MODV- and MMLV-induced mortality in SCID mice (Leyssen et al., 2001, 2003b; Charlier et al., 2002b). Infection with MODV of NMRI (Naval Medical Research Institute) mice that had received various degrees of immunosuppressive therapy revealed that immunopathological factors also contribute to disease progression but that direct virus-induced damage to the neurons is the principal cause of virus-induced mortality. As a consequence, antiviral therapy should have a beneficial effect on flavivirus encephalitis (Leyssen et al., 2003a).

The MODV and MMLV SCID models may be convenient for the study of antiviral strategies against flavivirus encephalitis, because MODV and MMLV are (i) highly pathogenic to (SCID) mice following peripheral inoculation, (ii) classified as biosafety level 2 pathogens [by the Subcommittee on Arbovirus Laboratory Safety (SALS) (http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s71.htm)], (iii) have the same overall organisation of the genome as flaviviruses that cause infections in man, and (iv) contain, in those genes that are considered to be interesting antiviral targets [i.e., the NS3 which encodes an NTPase/helicase and serine protease and the NS5 encoding a RNA-dependent RNA polymerase], the same conserved motifs as flaviviruses that are infectious to man (Charlier et al., 2002b; Leyssen et al., 2001, 2002, 2003a,b) (Table 3).

2.2.6.2. Hamster model. The Modoc virus is highly neuroinvasive in hamsters. Following intraperitoneal or intranasal inoculation with MODV, hamsters develop acute encephalitis. About 50–60% of the animals succumb during the acute phase of the infection, about 40% survive without neurological sequelae and the remaining 10% recover with obvious long-lasting neurological sequelae. The situation in hamsters that survive the acute phase of MOD encephalitis is reminiscent of that of patients who survived severe JE (Abe et al., 1998; Misra et al., 1994; Misra and Kalita, 1997; Richter and Shimojyo, 1961; Shoji et al., 1990; Steinhoff, 1996), SLE (Whitley, 1990), WN encephalitis (Ahmed et al., 2000) or TBE (Beer et al., 1999; Gunther et al., 1997; Kaiser, 1999; Kuntzer et al., 1995; Schellinger et al., 2000). In hamsters, viral RNA was detected during the acute phase of the disease in the brain, salivary glands, lungs and kidneys of infected hamsters. Perivascular cuffing, one of the characteristics of encephalitis caused by flaviviruses, was observed around small or medium-size blood vessels in the brain. A particular characteristic of the MODV/hamster model is that MODV-infected hamsters shed virus in the urine. This allows the evaluation of antiviral strategies over a period of several days in one and the same animal without having to use invasive sampling methods (Leyssen et al., 2001; Davis and Hardy, 1974) (Table 3).

2.3. Flaviviruses primarily associated with hemorrhagic fever

2.3.1. Yellow fever virus

2.3.1.1. Mouse model. Considerable variation in the neuropathogenicity of yellow fever virus for different strains of mice has been noted. Various strains of YFV killed young (4- to 5-week-old) BALB/c and TO mice within 2 weeks post-intracerebral inoculation. Following intranasal inoculation, only the Asibi virus, the French neurotropic vaccine, two out of three 17D vaccine substrain viruses (Brazil and Colombia) and some wild-type isolates were able to cause mortality (Barrett and Gould, 1986). Older mice were only susceptible to the infection when inoculated intracerebrally (Burke and Monath, 2001). Passage of the vaccine strain YFV17D in SCID and ICR mice resulted in a neuroinvasive YFV variant (SPYF) (Chambers and Nickells, 2001). This virus caused uniform mortality in young adult CD-1 mice following intracerebral inoculation of as little as 1 pfu of virus, replicated in mouse-brain tissue more rapidly than the non-neuroadapted virus, and resulted in higher peak titers of brain-associated virus and earlier death (Schlesinger et al., 1996). Also adult SCID mice proved very sensitive to

the neuroadapted virus, and developed a fatal encephalitis as early as 8 days post-intraperitoneal inoculation (Chambers and Nickells, 2001; Nickells and Chambers, 2003) (Table 3).

2.3.1.2. Hamster model. Following intraperitoneal inoculation with the virulent YFV strain (Jimenez), golden hamsters developed a high-titered viremia (lasting 5-6 days) and had abnormal liver function tests. The liver showed spotty necrosis as early as day 3 post-infection, later followed by steatosis and focally confluent necrosis. The spleen initially exhibited lymphoid hyperplasia, which was followed by lymphoid depletion and increased phagocytosis by splenic macrophages. Viral antigen was detected in the liver and the spleen. YFV antibodies appeared 4 or 5 days after infection. The mortality rate in YFV-infected hamsters was variable and was depending on the virus strain and the age of the animals (Xiao et al., 2001b). These clinical and pathologic changes in hamsters were very similar to those described in experimentally infected macaques and in fatal human cases of YF. In patients dying of YF, abnormalities have been described in multiple organs, most consistently in the liver (Monath, 1989). The liver is usually enlarged and has a mottled yellow appearance. Microscopically, necrosis of the hepatocytes has been observed. The spleen is usually enlarged, congested, and firm. In rhesus monkeys experimentally infected with YFV, the principal finding during the first 3 days is an eosinophilic necrosis of Kupffer's cells in the liver. Progressive degeneration and necrosis of hepatocytes became evident at about 3 days after infection and reached a peak on day 5. The necrosis correlated with the accumulation of viral antigen in the hepatocytes. The golden hamster YFV model may therefore be an excellent alternative for the non-human primate models for viscerotropic flavivirus infections (Tesh et al., 2001; Xiao et al., 2001b; Arya, 2001) (Table 3).

Besides mice and hamsters, also monkeys [e.g., *Saimiri sciureus* (squirrel monkeys), *Macaca mulatta* (rhesus monkeys)] have been used as a model for the study of potential antiviral drugs (such as interferon) (Arroyo et al., 1988; Scott et al., 1976).

3. Conclusion

Convenient small animal models that allow to rapidly assess the potential of novel strategies for the treatment or prevention of flavivirus infections may be instrumental to develop specific antiviral approaches for the treatment of flavivirus infections in man. Monkey models of flavivirus infections have been elaborated (for example, for YFV, DENV or JEV), but because of the cost involved and the restricted availability of monkeys, the number of studies that can be carried out using these animals is limited. Some flaviviruses (e.g., DENV, YFV) cause morbidity and mortality in mice, but only when inoculated intracerebrally and when virus strains are used that have been adapted by serial passage in the brain of suckling mice. Other flaviviruses (e.g., TBEV, JEV) cause infection in mice following peripheral inoculation, but these viruses require high containment laboratory facilities.

Flaviviruses cause a variety of diseases in man. Several flaviviruses cause severe neurological problems (e.g., JEV, TBEV). Others may cause severe hepatitis (YFV), shock syndrome (DENV) or hemorrhagic fever (YFV, DENV). Given the diverse pathology that different flaviviruses cause, there is a need for models that mimic these various clinical conditions.

Viruses such as the Modoc virus or the Montana Myotis leukoencephalitis virus (Leyssen et al., 2001, 2002, 2003b; Charlier et al., 2002a,b) cause a pathology in mice (and hamsters) that is reminiscent of flavivirus encephalitis in man. Both viruses are BSL-2 pathogens and have a genomic organisation and conserved motifs [in genes that encode antiviral targets] similar to flaviviruses that cause encephalitis in man. The MODV and MMLV models may therefore be particularly attractive for antiviral studies against flavivirus encephalitis.

Other mouse models mimic (to some extent) dengue hemorrhagic fever/dengue shock syndrome in man (Huang et al., 2000; Atrasheuskaya et al., 2003) and may therefore be useful as models for the study of prophylaxis and/or therapy against this viral diseases.

Golden hamsters inoculated with the Jimenez strain of YFV develop clinical and pathologic changes (such as abnormal liver function) that are very similar to those described in fatal human cases of YF (Tesh et al., 2001; Xiao et al., 2001b; Arya, 2001). The hamster/YFV model may therefore be an excellent alternative for the non-human primate models for viscerotropic flavivirus infections.

A modus operandi to evaluate the potential of novel molecules that have been proven to exert potent (and broad-spectrum) in vitro activity against flaviviruses, would be first to evaluate the activity in easy to manipulate rodent models such as the Modoc models in mice and hamsters. In such case where a molecule would appear sufficiently potent, its activity could next be assessed in other, but more difficult to manipulate rodent models such as those that require BSL-3 (or BSL-4) facilities. Molecules that, following evaluation in these rodent models, still prove sufficiently potent, may then be studied in relevant monkey (or other animal) models, if available.

The intense search for selective inhibitors of the hepatitis C virus (HCV), a virus that belongs, like flaviviruses, to the family of the Flaviviridae, will hopefully also lead to the discovery of selective inhibitors of flavivirus replication. It will be important to assess the efficacy of such compounds in reliable and relevant small animal models before persuing further preclinical studies in monkeys or other larger animals.

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