

Flavivirus Neurotropism, Neuroinvasion, Neurovirulence and Neurosusceptibility: Clues to Understanding Flavivirus- and Dengue-Induced Encephalitis

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1. Introduction

Viral infections of the nervous system (NS) can be caused by many types of viruses, including rhabdoviruses, alpha and beta herpes viruses, retroviruses, picornaviruses, arenaviruses and flaviviruses (van den Pol, 2006). The replication of these viruses can occur both in neurons and in non-neuronal cells and each type of cell responds differently (Griffin, 2003). The final result of these infections is the alteration of function of the nervous system.

Flaviviruses are single-stranded positive sense RNA viruses of epidemiological and neurological importance because the majority of them infect the NS, causing severe damage to its function (Figure 1) (Lindenbach et al., 2007). The flaviviruses that most frequently infect nervous tissue are Japanese encephalitis virus (JEV), West Nile virus (WNV), Murray Valley encephalitis virus (MVEV) and tick-borne encephalitis virus (TBEV). However, other members of this family, such as yellow fever virus (YFV) and dengue virus (DENV), which preferentially infect hepatocytes and immune cells like monocytes and macrophages, can acquire the capacity to enter and infect nervous tissue (Misra et al., 2006).

Infection with flaviviruses occurs via an interaction between viral envelope (E) proteins and molecules on the cellular surface that act as receptors that promote endocytosis of the virus (Chambers et al., 1990; Lindenbach et al., 2007; van der Schaar et al., 2007, 2008). This initial interaction partially defines the virus tropism; however, the mechanisms that determine and promote infection of nervous tissue with neurotropic flaviviruses are not completely understood (Chambers & Diamond, 1999; McMinn, 1997). Furthermore, nervous tissue infected with DENV is of particular interest because although this virus is not neurotropic, it can induce alterations in nervous system function that are being reported with increasing frequency. In some cases, virus-specific IgMs have been isolated from the cerebrospinal fluid, which suggests the presence of the virus in the NS (Domingues et al., 2008; Lum et al., 1996).

In severe cases of dengue fever, neurological alterations including encephalitis, encephalomyelitis, transverse myelitis, flaccid paralysis, Guillain-Barre Syndrome, cerebrovascular accident and behaviour disorders have been reported (Domingues et al.,

2008; Mathew & Pandian, 2010; Misra et al., 2006; Solomon, 2003, 2004). Frequently, neurological signs manifest as a consequence of viral infection in organs such the liver (encephalopathies) (Gulati & Maheshwari, 2007; Row et al., 1996). Despite the fact that little is known about the mechanisms that favour DENV infection of nervous tissue (Chien et al., 2008; Kumar et al., 2008; Malavige et al., 2007), it has been postulated that the individuals' age, genetic background and immune status, in addition to the viral serotype and genotype, may explain both the ability of the virus to infect the NS and the appearance of neurological manifestations as a result of this virus infection. This chapter will review the interactions between nervous tissue and certain flaviviruses, including DENV, such as neuroinvasion, neurotropism, neurovirulence and neurosusceptibility.

A. Genomic RNA of Flavivirus



B. Polypeptide Translation



C. Organization of structural proteins in flavivirus

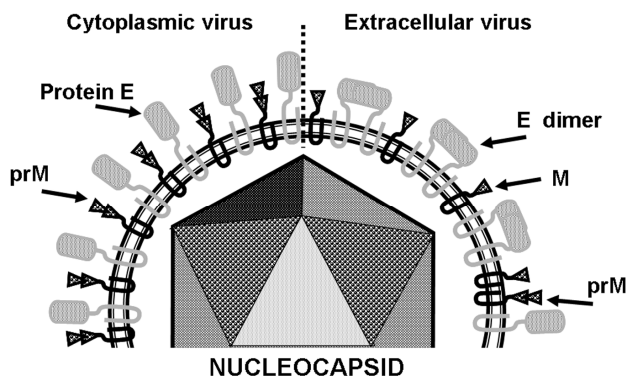


Fig. 1. Schematic organization of genome and polyprotein of a flavivirus. A. The flaviviruses are enveloped single strand RNA viruses with a unique open reading frame coding for both structural and non structural genes. B. Diagram of entire translated polyprotein which must be cleaved by viral and cellular proteases to release mature and active proteins. C. Schematic representation of final disposition of structural proteins in the virions. After proteolytic cleavage of prM, glycoprotein E exposes its homodimerization domains to activate the cell-binding site. Core protein specifically encapsidates the recently synthesized genomic RNA to form the nucleocapsid.

2. Neuroinvasion

Neuroinvasion is the ability of viruses to enter nervous tissue and cause neurological alterations. The majority of viruses in the Flavivirus genus are transmitted via the bite of an

arthropod vector (mosquito or tick), and once inoculated in the dermis, these viruses spread to infect target cells such as dendritic cells or monocytes/macrophages or enter directly into the lymph nodes, muscles, liver, spleen or nervous system via nerve endings (Chambers & Diamond, 2003; McMinn, 1997). In some cases during infection with these viruses, the blood-brain barrier (BBB) is disturbed as a result of cytokines and chemokines that favour the entry of WNV and JEV into nervous tissue (Chambers & Diamond 2003; Chaturvedi et al., 1991) (Figure 2).

2.1 The blood-brain barrier disruption and axonal transport

The BBB is formed by specialised endothelial cells, surrounded by a basal lamina, pericytes, astrocytes and neurons that together form the neurovascular unit (NVU). This structure acts as a physical and metabolic barrier that restricts the type of nutrients and molecules that can enter the cerebral parenchyma (Banerjee & Bhat, 2007; Calabria & Shusta, 2006; Cardoso et al., 2010). Inter-endothelial junctions formed by membrane proteins present at tight junctions (claudins, occludins and Junctional Adhesion Molecules (JAM) and adherens junctions (cadherins and catenins) filter nutrients and metabolites and regulate the passage of immune cells into nervous tissue (Cardoso et al., 2010).

However, during infection with neurotropic flaviviruses such as WNV, JEV and MVEV, the over-expression of cytokines, such as tumour necrosis factor-alpha (TNF-alpha), or enzymes, such as matrix metalloproteinase (MMP), affects the permeability of the endothelium and permits the entry of viruses into the cerebral parenchyma (Chambers & Diamond, 2003). Wang et al. (2004) reported that during WNV infection, the over-expression of TNF-alpha and interleukin 6 (IL-6) affects the integrity of the BBB because they alter expression of the proteins responsible for inter-endothelial junctions (Wang et al., 2004). Additionally, MMP enzymes digest the basal lamina, weakening the interactions between endothelial cells and other elements forming the NVU (Cardoso et al., 2010; Petty & Lo, 2002; Wang et al., 2004), favouring the entry of viral particles or infected leukocytes into the cerebral parenchyma and facilitating the spreading and replication of the virus in nervous tissue (Wang et al., 2004).

Additionally during infection, endothelial cells are activated and overexpress cellular adhesion molecules that favour the transmigration of immune cells into the cerebral parenchyma, such as E-selectin, VCAM-1 and ICAM-1 (Shen et al., 1997; Verna et al., 2009). For example, the overexpression of ICAM-1 promotes the adhesion and diapedesis of infected and activated leukocytes that can enter and alter the brain. These cells can also amplify the infection, acting as *Trojan horses* that introduce viral particles into nervous tissue (Ben-Nathan et al., 1996; Cardosa et al., 1986; King et al., 2007). Infection with flaviviruses and the signalling induced by some cytokines expressed extraneurally during such an infection can activate macrophages and microglial cells. These cells then acquire an antigen-presenting phenotype and produce and spread pro- and anti-inflammatory molecules such as IL-6, IL-1 β , IL-10, TNF-alpha, type I and II interferon (IFN) and the monocytes chemotactic protein (MCP-1) in the brain microenvironment (Goshal et al., 2007), which promotes the disturbance of the endothelium and increases and sustains the activation of glial cells, promoting the infiltration of leukocytes (Muñoz-Fernández & Fresno, 1998) Another aspect that contributes to the disturbance of the BBB and the transport of flaviviruses into the cerebral parenchyma is the infection of endothelial cells (Avirutnan et al., 1998), which allows replication of the virus and its subsequent movement toward the cerebral parenchyma (Liu et al., 2008; Lopes et al., 2007; Mathur et al., 1992; Mishra et al., 2009) (Figure 3).

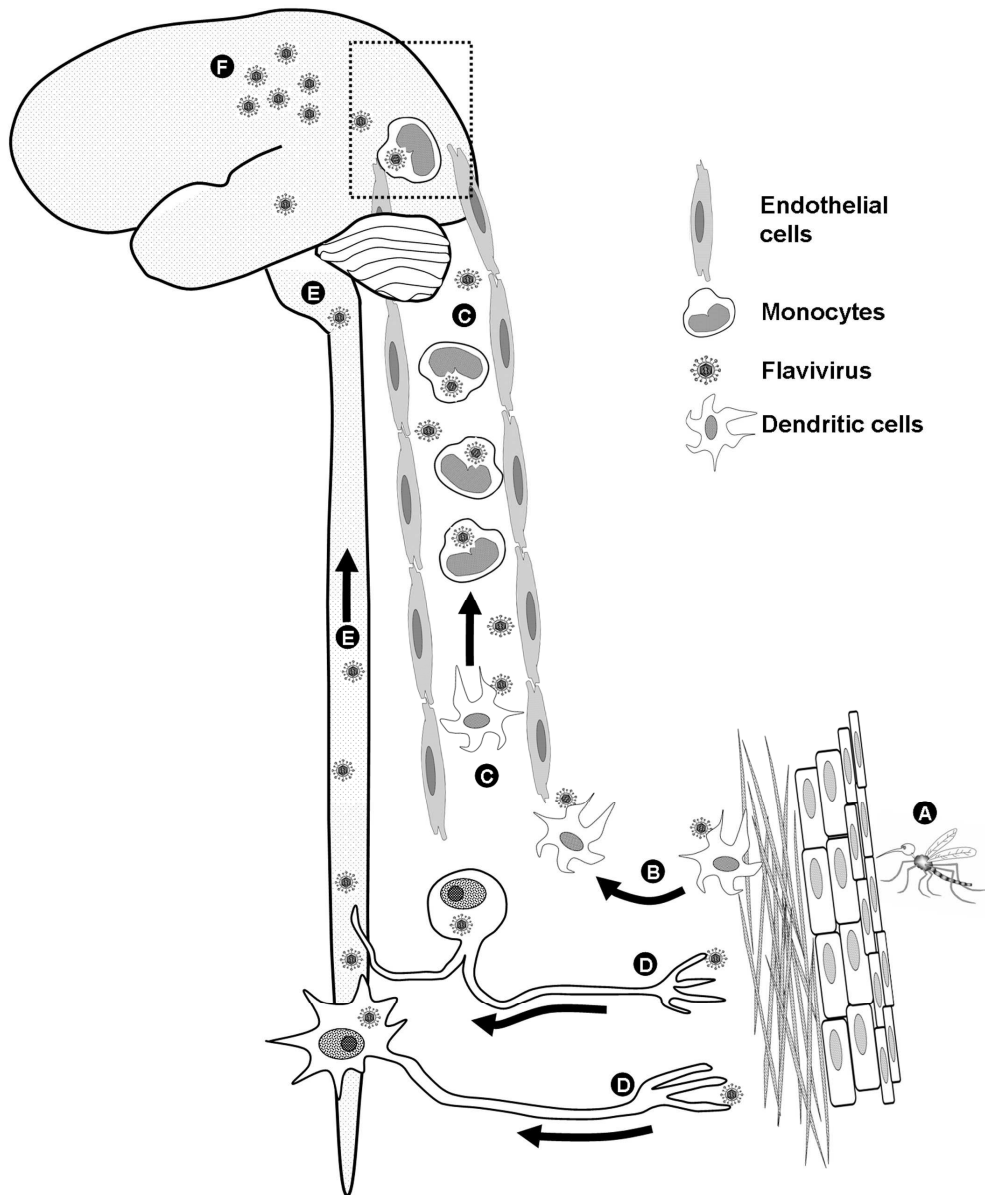


Fig. 2. Mechanisms for flavivirus entry into nervous system during neurologic disease. Neurotropism could be explained by direct binding of virus with neurons or ability of neurons to replicate the virus, while neuroinvasión and neurovirulence depends on ability of virus to enter to CNS and disrupt the brain architecture or function. A. - Flavivirus inoculation by an arthropod bite in the dermis. B. - Dendritic cells or Langerhans cells take the inoculated virus and migrates to lymph nodes to infect other immune cells. C. - Virus-

free or cell-associated virus spreading using hematogenous pathway to enter CNS and infect neurons and glia. D. - Virus capture and spreading by sensory and motor peripheral fibers. Virus is transported by retrograde axonal transport to CNS, and E. - spread following connecting neurons. F. - Then; virus can infect neighborhood neurons affecting its metabolism and function. During extraneural infection, immune cells produce cytokines and chemokines, which induce adhesion molecules expression in brain endothelial cells, favouring rolling of monocytes and macrophages. In addition to cells, the over expression of pro-inflammatory mediators and proteolytic enzymes result in an increase in blood-brain barrier permeability (see explanation of inset box in Figure 3).

However, infection and the damage to endothelial cells *in vivo* is not always evident, and Liou and Hsu (1998) demonstrated that JEV passes through brain endothelial cells via transcytosis (Liou & Hsu, 1998), suggesting that flaviviruses can exploit a diversity of mechanisms to penetrate nervous tissue. Other routes used by flaviviruses to enter the NS include the hematogenous route and axonal transport. The haematogenous route is the most likely dispersion route of various viruses, including flaviviruses, because these viruses, after being inoculated by mosquitoes or ticks, infect monocytes/macrophages, dendritic cells and Langerhans cells, which then transport viruses to a second cell type, such as epithelial, endothelial, fibroblast or muscle cells (Chambers & Diamond 2003; Lindenbach et al., 2007).

WNV, JEV and TBEV also utilise axonal retrograde or anterograde transport in olfactory epithelial neurons and motor neurons to penetrate and spread within the central nervous system (CNS) (Charles et al., 1995; Monath et al., 1983; Ramos et al., 1994; Sriurairatna et al., 1973), and peripheral nervous system (PNS) (An et al., 2003; McMinn et al., 1996; Samuel et al., 2007a; Silvia et al., 2003). Samuel et al. (2007b) using *in vitro* and *in vivo* infection models demonstrated that WNV enters to CNS and is transported efficiently using axonal transport in spinal medullary neurons and superior cervical ganglion neurons. Additionally, they demonstrated that in a hamster model, after viral inoculation and sciatic nerve transection, animals exhibited neurological alterations such as paralysis and prostration, suggesting that WNV utilises both the nervous and hematogenous routes to penetrate and replicate in nervous tissues (Samuel et al., 2007b).

2.2 DENV neuroinvasion

With regard to infection of the NS by DENV, it has been reported that this virus can infect NS cells *in vitro* and *in vivo* and could use axonal transport to spread inside the brain. Moreover, it has been demonstrated that *in vivo* infection with DENV can alter the integrity of the BBB (Chaturvedi et al., 1991), which has been associated with high levels of MMP-9 in plasma. This enzyme can degrade the basal lamina of the NVUs and facilitate the free passage of the virus and infected leukocytes into the cerebral parenchyma (Luplertlop et al., 2006). In animal models, NS infection by DENV has been reported after the virus was tissue-adapted, as reported by Cole and Wisseman (1969) and Sriurairatna et al. (1973). These authors achieved infection and virus production in tissue, accompanied by neurological signs associated with infection, such as paralysis of the posterior limbs. This infection was achieved after adapting a DENV strain via numerous passages in mice brains (Cole & Wisseman, 1969; Sriurairatna et al., 1973).

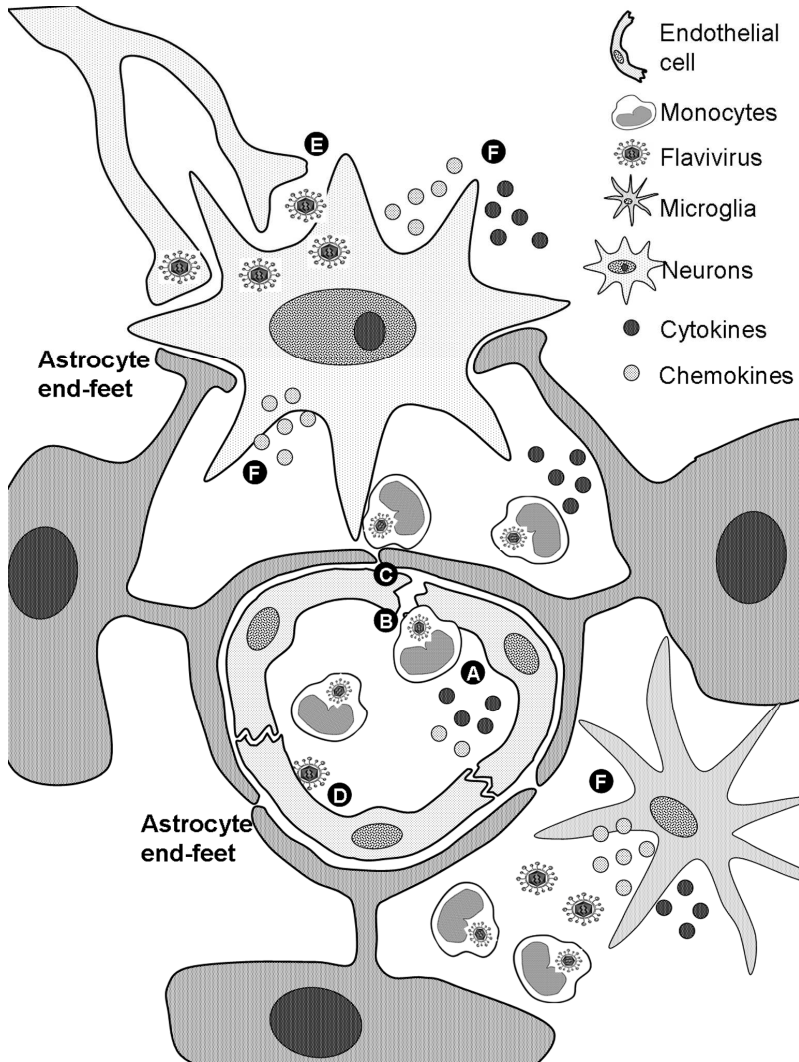


Fig. 3. Diagram of a neurovascular unit, a capillary enclosed in astrocyte end-feet during a nervous system infection by a flavivirus. A. - Infected and non-infected immune cells secretion of cytokines and chemokines. B. - Disruption of tight junctions that seal the pathway between the capillary endothelial cells, caused by cells and inflammatory mediators. C. - Transcytosis of infected cells to brain parenchyma. D. - Direct infection of brain endothelial cells by flavivirus. E. - Virus neuronal infection by neighbor infected neurons. F. - Secretion of cytokines and chemokines by infected neurons and glial cells, causing change of structure and function of nervous cells.

Desprès et al. (1996 and 1998) also reported nervous tissue infection in 2-day-old mice after viral adaptation of a DENV-1 strain in nervous tissue and mosquito cells. This model of

neuroinfection was the first to suggest that neurological alterations exhibited by infected animals, such as paralysis of posterior limbs, are principally associated with the death of infected neurons (Desprès et al., 1996, 1998). Another model of nervous tissue infection was developed by An et al. (2003), who infected young adult SCID mice (severe combined immunodeficiency) with a non adapted DENV-2 strain; these animals developed high viral titres in their nervous tissue, and viral particles were observed in spinal medullary motor neurons, axons and ependymal cells (An et al., 2003), using transmission electron microscopy, which suggests that DENV can penetrate and infect CNS and PNS neurons using axonal transport. Finally, it has been reported that DENV, regardless of whether it is adapted to nervous tissue, can infect a low proportion of primary neurons or cell lines *in vitro* (Desprès et al., 1996; Imbert et al., 1994; Ramos et al., 1994, 1998), thus demonstrating DENV weak neurotropism.

This evidence demonstrates that neuroadapted DENV, can infect the brain and can utilise axonal transport to enter and spread throughout the nervous system (An et al., 2003; Desprès et al., 1998; Liou & Hsu, 1998; Lum et al., 1996), Nevertheless, this virus continues to be classified as a non-neurotropic virus, and the elements that confer the capacity to enter, infect and spread in nervous tissue in this virus are completely unknown.

3. Neurotropism

The ability of some viruses to infect and replicate in neurons is called neurotropism and is determined by viral and cellular factors. Mostly virus determinants are associated with envelope glycoprotein gene mutations that favour interactions between the virus and molecules on the neuron surface. These interactions promote the fusion of the virus with the plasma membrane and can also trigger endocytosis or transcytosis of the virus. A well-known example of viral neurotropism is that mediated by glycoprotein G of the rabies virus (a highly neurotropic virus), which interacts with the neurotrophin low-affinity receptor, the neural cell adhesion molecule or the nicotinic receptor present in muscular and neuron cells to infect cells. The interaction of rabies virus protein G with some of these molecules promotes virus entry and replication in the nervous system (Lafon, 2005).

3.1 Viral and cellular proteins

The virus and cell interactions occur first with molecules that act as low-affinity receptors to bring virus closer to membrane, and then viral co-receptors bind to proteins on the viral surface and promote infection, which can be mediated by endocytosis or through the fusion of membranes such that the nucleocapsid is released into the cytoplasm. The use of co-receptors is common in many different viruses. The human immunodeficiency virus uses co-receptors to infect T lymphocytes and macrophages via the CD4 receptor and the CCR5 and CXCR4 molecules (Moiser, 2009).

In flaviviruses, the envelope protein (E) is the principal component of the virion surface. It participates in the recognition and subsequent binding to the receptor and the fusion of the virus with the cell membranes (Lindenbach et al., 2007). This protein is formed by three beta-barrel domains known as domains I, II and III, and these last two are responsible for interacting with putative receptor molecules (Pastorino et al., 2010). Variations in the amino acid sequence in domains II and III, which are associated with the lack of proofreading

activity of the flavivirus RNA polymerase, bear directly upon the changes of viral tropism and can promote the neurotropism of flaviviruses including DENV (Lee et al., 2006a).

The molecules that have been reported as possible receptors for DENV, JEV, WNV and TBEV in different cell populations include ICAM-3 (Jindadamrongwech & Smith, 2004), CD209 (DC-SIGN) (Tassaneetrithep et al., 2003), DC-SIGNR (Davis et al., 2006), integrins (Chu & Ng, 2004), the mannose receptor (Miller et al., 2008), HSP70 and HSP90 (Das et al., 2009; Reyes del Valle et al., 2005), the laminin receptor (Tio et al., 2005) and heparan sulphate (HS) (Germi et al., 2002) among others (Barba-Spaeth et al., 2005; Upanan et al., 2008). It seems that HS favours the attraction and recruitment of viral particles to the cellular surface, thus favouring the direct entry of the virus or interaction with a second receptor molecule (Germi et al., 2002; Lee et al., 2002, 2004, 2006b). Additionally, in human (SK-N-SH) and murine (N1E-115) neuroblastoma cell lysates, the presence of a 65 kDa protein that binds specifically to DENV-2 has been reported (Ramos et al., 1997), and these findings suggest that neuronal cells express a receptor that permits the binding of the virus with these cell membranes; however, the characterisation of this protein has not been reported.

As was previously stated, the E flavivirus protein partially determines cellular tropism. However, the molecular determinants that promote the entry into susceptible cells are not well known, and the mechanisms that define their neurotropism are even less clear (Lobigs et al., 1990). So far, the RGD Motif (Arg-Gly-Asp), present in the E protein of JEV, YFV, TBEV and WNV, has been identified as being responsible for promoting the interaction between these viruses and integrins present on the surface of susceptible cells. This was demonstrated by modification of position 390 of the E protein of MVEV, which changes the tropism and virulence of this virus. Thus, this amino acid motif is proposed as the main site of interaction between flaviviruses and their viral receptors (Becker, 1990; Lee & Lobigs, 2000; Lobigs et al., 1990). However, the RGD motif has not been identified in the E protein of DENV, which suggests that this virus possesses different domains or mechanisms for interacting with receptor molecules.

3.2 DENV neurotropism

Various authors have reported that during *in vitro* or *in vivo* passages associated with the adaptation of DENV, mutations occur throughout the genome, primarily in glycoprotein E, which seemingly confers neurotropism and the ability to enter nervous tissue and cause neurological alterations. For example, the Glu₁₂₆Lys change in the DENV-2 E protein changed the virus tropism and conferred the capacity to infect nervous tissue (Gualano et al., 1998). Similarly, the mutations Asp₃₉₀His and Phe₄₀₂Leu in DENV-4 E protein conferred a neurotropic and neurovirulent phenotype on the virus (Bray et al., 1998; Kawano et al., 1993; Sanchez & Ruiz, 1996). These findings suggest that for DENV to acquire a neurotropic phenotype, certain variations must occur in the sequence located in specific regions of the E protein (Desprès et al., 1996; Lee et al., 2006a). However, mutations in non-structural viral proteins could also determine the success of the infection, particularly the replication of the virus in neurons (Duarte dos Santos et al., 2000). Consequently, it should be determined whether the neurotropism of adapted DENV depends on the viral serotype used and the type of cell to which the virus is adapted. Additionally, the mechanisms of DENV transport and dispersion throughout the nervous tissue and whether these mechanisms depend on

changes in the viral genome acquired during the process of neuroadaptation should be evaluated.

4. Neurovirulence

Neurovirulence is the capacity of viruses to cause disease and alterations in the nervous system and can be affected by both viral and host-related factors. The viral factors that affect neurovirulence are viral serotype and genotype. Beasley et al. (2002) reported that genotypes I and II of WNV, which are very similar in sequence, cause different neurological alterations in mice and hamsters (Beasley et al., 2002).

4.1 Serotypes and genotypes

The dengue serocomplex is formed by 4 viral serotypes that possess a high genome homology and all cause dengue symptoms. However, it has been demonstrated that the genomic differences among DENV serotypes and genotypes induce clinical manifestations of the disease that vary in intensity, as has been shown for DENV-2 and DENV-3, which have been associated mainly with haemorrhagic symptoms and cases of severe dengue in some patients (Clyde et al., 2006; Tsia et al., 2009).

For example, the best-studied genotypes of DENV-2 are the Asian and American genotypes. When the Asian strain started circulation in the American continent, it was caused serious dengue outbreaks with haemorrhagic symptoms in patients with primary infections who were from Central and South American countries (Clayde et al., 2006). The Asian genotype is frequently associated with severe dengue and haemorrhagic symptoms in Asian patients, and experimentally, this genotype is more virulent and replicates with higher efficiency in macrophages, while the American genotype is associated with signs of dengue fever and its replication is slower in cultured macrophages (Barreto dos Santos et al., 2002; Guzmán et al., 2002a; Rico-Hesse et al., 1997).

These differences could partially explain the changes in symptoms exhibited by patients infected with DENV. When the genotype sequences were compared, significant differences were found in the 5' and 3' untranslated regions (UTR) of the genomic RNA, and it was observed that the 3' UTR of the Asian genotype generates secondary structures that permit better interaction with the viral RNA polymerase and enhance its processivity. This difference could explain the efficiency of virus replication and virus production in infected cells with this genotype, which in turn could be related to the inefficiency of the immune system to control and eliminate this virus (Cologma & Rico-Hesse, 2003; Leitmeyer et al., 1999).

Additionally, it has been reported that genotype I of DENV-3 can induce different symptoms in infected mice, when was intracerebrally inoculated. An effective infection was observed with high viral titer detected in tissue. Infiltration of monocyte cells into the cerebral parenchyma was also detected, as was the appearance of neurological symptoms such as meningo-encephalitis and paralysis associated with neuronal degeneration. In contrast, intracerebral inoculation of mice with genotype III induced a less intense immune response, with less tissue damage and low viral production (Ferreira et al., 2010), confirming that differences among genotypes and serotypes can be related to flavivirus virulence.

Beyond viral factors, the neurovirulence caused by flaviviruses can be related to the type of immune response that the individual generates against infection at the local and systemic levels. This response is similar among flaviviruses, although DENV and YFV mainly induce alterations in vascular permeability and in coagulation (Avirutnan et al., 2010). The immune response that occurs in nervous tissue during flavivirus infection varies in intensity and can support the control and clearance of the virus and establish a neuroprotective state that stimulates the repair of tissue damaged by the infection (Griffin, 2003). To promote virus clearance, monocytes and lymphocytes enter the cerebral parenchyma, attack infected cells, and release soluble mediators, which stimulate and maintain the local immune response activating astrocytes, microglia and the cerebrovascular endothelium. Additionally, the infected or damaged neurons themselves can express and release some of these mediators (Chakraborty et al., 2010), amplifying the local immune response.

4.2 The host factor: Immune and nervous system

As was mentioned above, the activated cerebrovascular endothelium can facilitate the passage of T and B lymphocytes and macrophages and allow the leakage of soluble factors and toxins that increase inflammation and damage the cerebral parenchyma, causing neuron death (Less et al., 2006; Lin et al., 2002; Wrona, 2006). Inflammation of nervous tissue is associated with the activation of astrocytes and microglia, as indicated by morphological changes and changes in the expression profile of adhesion molecules, cytokines and interleukins (TNF- α , IL-1 β , IL-6, IL-10, IFNs, MCP-1 and TGF- β), which combined with factors secreted by infiltrating immune cells, can increase the nervous system damage (Muñoz-Fernández & Fresno, 1998). The activation of glial cells is partially due to their infection by flaviviruses. For example, astrocytes infected with WNV express the chemokine CXCL10 and other neuroinflammatory and neurotoxic molecules that can increase nervous system injury and induce the death of both infected and uninfected neurons. These data suggest that the activation of glial cells depends on viral replication and that the signalling induced by some inflammatory mediators within the nervous tissue can increase the neuropathogenesis caused by WNV (Van Marle et al., 2007) and other flaviviruses.

Immune cells that infiltrate nervous tissue are mainly CD4⁺ and CD8⁺ T lymphocytes and macrophages. CD4⁺ lymphocytes producing IL-12 stimulate the cytotoxic activity of CD8⁺ lymphocytes that arrive to the nervous tissue. These lymphocytes secrete proinflammatory mediators such as IFN- γ , TNF- α and IL-6 that alter tissue homeostasis when expressed consistently during the infection (Chaturvedi et al., 2000; Sánchez-Burgos et al., 2004; Swarup et al., 2007). Additionally, CD8⁺ lymphocytes embedded in the tissue promote the death of both infected and uninfected cells via the release of perforin and granzymes and the expression of Fas ligand (FasL) (Courageot et al., 2003; Marques-Deak et al., 2005; Mellor & Munn, 2006; Rempel et al., 2004, 2005). Lastly, infiltrating macrophages modulate the type of immune response that occurs in the tissue during infection and can clear free viral particles and infected and damaged cells present in the tissue, although these cells can also seemingly promote the entering of some flaviviruses, acting as *Trojan horses* by releasing viral particles within the nervous tissue (Chaturvedi, 2006; Chaturvedi et al., 2006).

Finally, neurotropic flaviviruses as well as non-neurotropic flaviviruses preferably infect neurons *in vitro* and *in vivo* (Chambers & Diamond, 2003; Johnson & Roehring, 1999; Samuel & Diamond, 2006; Shrestha et al., 2003). Nevertheless, it has been reported that other

nervous tissue cells such as oligodendrocytes, astrocytes and microglial cells (Chen et al., 2000; Jordan et al., 2000) may be susceptible to infection. The cellular, metabolic and molecular factors that increase the susceptibility of neurons to flaviviruses are unknown. Additionally, it has been reported that infection with some flaviviruses, including DENV, induces death in infected neurons. This response may be mediated by TNF-alpha, the Fas/FasL complex or the release of cytochrome c and the presence of free radicals; during this process, caspases 3, 8, and 9 have been found to be activated (Courageot et al., 2003; Marianneau et al., 1998; Samuel et al., 2007a). Thus, cell death in nervous tissue, such as neurons, has been associated with the development of neurological alterations resulting from infection.

5. Neurosusceptibility

Neurosusceptibility refers to the vulnerability of a host to neurological alterations during an infection with neurotropic viruses. This vulnerability can be affected by the age, species, immune status and genetic background of the individual.

5.1 Age

Using animal models, it has been demonstrated that physiological immaturity increases the susceptibility of the nervous system to WNV and JEV infection (Ogata et al., 1991; Weiner et al., 1970). For example, JEV infects neurons in the cortex, hippocampus and brainstem of 2-day-old mice, but the areas susceptible to infection and the numbers of infected neurons diminish as age increases. This resistance to infection is maintained, even if previously-infected neonatal neurons are implanted in animals greater than 14 days old. Similarly, the mortality of rats infected with JEV is 100% when they are inoculated between 2 and 12 days after birth but diminishes to 50%, 8.3% and 0% when they are inoculated at 13, 14 and 17 postnatal days. These results demonstrate that the neuronal and physiological maturity of nervous tissues is determining factors in favouring infection and neuronal alteration (Ogata et al., 1991; Weiner et al., 1970).

With respect to DENV-2 infection, Guzmán et al. (2002b) reported that children between the age of 3 and 4 years old were more vulnerable to developing symptoms of dengue compared to older children and adults (Guzmán et al., 2002b). This vulnerability is due principally to the type of response that neonates generate against viral, bacterial, fungal and parasitic infections (Maródi, 2006). Some clinical reports demonstrate that neonatal immunity is predominantly of the Th2 type, which specifically stimulates immune tolerance and inhibits the Th1 type response, which in turn activates immune cells to control and eliminate pathogens.

This tolerance and ineffectiveness in young individuals is related to the type of cytokines that are released and circulate before and after infection, such as the immunomodulatory molecule IL-10 that negatively regulates the activation of cells such as macrophages, NK cells and T and B lymphocytes. This hypo-reactivity of antigen-presenting cells causes them to inefficiently recognise and present viral or bacterial antigens. Additionally, in neonates, the absence of specific antibodies against microorganisms and the low level of production of molecules like IFN-gamma and TNF-alpha further reduce the activation of Th1 lymphocytes (Kemp & Campbell, 1996; Maródi, 2006; Wilson et al., 1999), and thus the cytotoxic activity and pathogen control exhibited by lymphocytes is not established.

5.2 Genetic background

The species and genetic background of vectors and hosts are other determinants that can favour the dispersion of flaviviruses and neuroinfection. The enzootic life cycle of flaviviruses includes vectors and reservoirs such as birds, monkeys or other wildlife, as well as humans. Flavivirus vectors can include mosquitoes of the *Aedes* and *Culex* genera and ticks of the *Ixodes* genus, which transmit TBEV (Lindenbach et al., 2007). The known reservoirs for flaviviruses are birds and small mammals, which suggests that there are some species-specific characteristics that restrict the transmission of these viruses. These ecological restrictions are evident during DENV infection in some experimental infection models in mice and monkeys, which reproduce some signs of disease that manifest in infected humans. Additionally, in these models, certain symptoms associated with infection are exhibited that are uncommon in infected humans, such as neurological alterations (Tan et al., 2010), which render interpretation of the data more difficult.

Murine models commonly used to reproduce certain symptoms associated with DENV infection are mice models such as SCID (Lin et al., 1998), AG129 (lacking functional IFN- α/β and- γ receptors) (Johnson & Roehring, 1999; Williams et al., 2009; Tan et al., 2010) and NOD/SCID (non-obese diabetic/severe combined immunodeficient) (Bente et al., 2005; Huang et al., 2000; Mota & Rico-Hesse, 2009), which upon being infected by DENV develop some signs of disease such as haemorrhage, thrombocytopenia and plasma leakage (Shresta et al., 2006). Nevertheless, while these models have increased our understanding of some of the cellular and molecular mechanisms involved in the development of the haemorrhagic signs observed during infection, their interpretation should be tentative given that these animals present an incomplete immune response to the virus due to their modified genomes.

With these differences in mind, other models have been established using immunocompetent animals such as C57BJ/C, ICR, A/J (Shresta et al., 2004) and Balb/C (Barreto et al., 2007) mice, which present robust immune responses to the virus, and possibly are less susceptible to infection as a result. However, these animals contract the virus and develop symptoms when infected with mouse cell- or tissue-adapted DENV or following intravenous or intracerebral inoculation with high viral titres in suckling or young adult mice (Yauch & Shresta, 2008; Wu-Hsieh et al., 2009). These models allow the acquisition of other data that allow a different understanding of the molecular mechanisms associated with DENV immunopathogenesis. Nevertheless, independent of the strain of animals employed, one must keep in mind that these models are experimental tools that so far only allow the *in vivo* reproduction of some symptoms of the very complicated disease induced by DENV.

6. Conclusion

The infection and pathogenesis caused by neurotropic flaviviruses is a product of a series of complex interactions between the virus and nervous tissues and is affected by viral diversity and the host's immune response and susceptibility. Therefore, it will be necessary to perform new studies with new experimental strategies to expand our knowledge and understand the interactions between flaviviruses and nervous tissue. It will be necessary to identify those factors affecting DENV and the nervous system that favour neuroinfection

and the increasingly frequent appearance of neurological symptoms stemming from this virus. The study of this phenomenon will provide information that permits an understanding of viral pathogenesis that is of great importance for public health in tropical countries. In addition, this proposal will uncover new strategies for antiviral and vaccine research that will be useful for fighting DENV.

7. Acknowledgment

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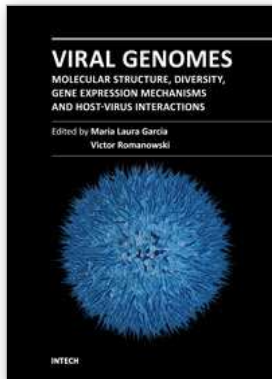
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**Viral Genomes - Molecular Structure, Diversity, Gene Expression
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Viruses are small infectious agents that can replicate only inside the living cells of susceptible organisms. The understanding of the molecular events underlying the infectious process has been of central interest to improve strategies aimed at combating viral diseases of medical, veterinary and agricultural importance. Some of the viruses cause dreadful diseases, while others are also of interest as tools for gene transduction and expression and in non-polluting insect pest management strategies. The contributions in this book provide the reader with a perspective on the wide spectrum of virus-host systems. They are organized in sections based on the major topics covered: viral genomes organization, regulation of replication and gene expression, genome diversity and evolution, virus-host interactions, including clinically relevant features. The chapters also cover a wide range of technical approaches, including high throughput methods to assess genome variation or stability. This book should appeal to all those interested in fundamental and applied aspects of virology.

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