

Advances and new insights in the neuropathogenesis of dengue infection

Avanços e novos aspectos na neuropatogênese da dengue

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ABSTRACT

Dengue virus (DENV) infects approximately 390 million persons every year in more than 100 countries. Reports of neurological complications are more frequently. The objective of this narrative review is to bring up the advances in the dengue neuropathogenesis. DENV can access the nervous system through blood-brain barrier disturbance mediated by cytokine. The blood-cerebrospinal fluid (CSF) barrier seems to be also involved, considering the presence of the virus in the CSF of patients with neurological manifestations. As for neurotropism, several studies showed the presence of RNA and viral antigens in brain tissue and CSF in humans. In murine model, different virus mutations were associated to neurovirulence. Despite the advances in the dengue neuropathogenesis, it is still necessary to determine a more appropriate animal model and increase the number of cases of autopsy. The detection of neurovirulence markers may contribute to establish a prognosis, the disease control and vaccine development.

Keywords: dengue, neuropathogenesis, neurotropism, neurovirulence.

RESUMO

O vírus da dengue (DENV) infecta anualmente cerca de 390 milhões de indivíduos em mais de 100 países. Complicações neurológicas estão se tornando frequentes. O objetivo desta revisão narrativa é abordar os avanços sobre neuropatogênese na dengue. O DENV invade o sistema nervoso central através do distúrbio da barreira hemato-encefálica, mediado por citocina. A barreira hemato-líquórica (LCR) parece também estar envolvida, considerando a presença do vírus no LCR. Estudos demonstraram RNA e antígenos virais no tecido cerebral e LCR de indivíduos infectados pelo DENV, confirmando o neurotropismo viral. Em modelo murino, diferentes mutações virais foram associadas a neurovirulência. Apesar dos avanços no conhecimento da neuropatogênese da dengue, ainda são necessários a determinação de um modelo animal mais adequado e aumento do número de casos de autópsia. A determinação de marcadores de neurovirulência pode contribuir para o estabelecimento de prognóstico, controle da doença e no desenvolvimento de vacina.

Palavras-chave: dengue, neuropatogênese, neurotropismo, neurovirulência.

Dengue virus (DENV) is an arbovirus transmitted mainly by two species of mosquitoes: *Aedes aegypti* and *A. albopictus*. This RNA virus belongs to *Flavivirus* genus of *Flaviviridae* family and infects approximately 390 million persons every year in more than 100 countries¹. Its genomes comprises a ~11kb long single strand positive-sense RNA that encodes a polyprotein precursor that is cleaved by virus and host cell proteases. Thus, this polyprotein yields the structural proteins (E, M and C) as also the non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5)². There are four different DENV genotypes described (DENV1, DENV2, DENV3 and DENV4)¹. All

of them can cause disease in humans. A possible fifth serotype has recently been detected, but its importance as disease agent is not clear¹.

DENV can cause a wide spectrum of clinical manifestation, ranging from a self-limiting febrile syndrome (dengue fever, DF), severe hemorrhagic syndrome (DHF) and severe shock syndrome (DSS). In 2009, WHO proposed a new criteria for dengue classification according to levels of severity: dengue without warning signs; dengue with warning signs and severe dengue. Abdominal pain, liver enlargement, lethargy, persistent vomiting, fluid accumulation, mucosal

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bleeding as also as increasing hematocrit with concomitant decreasing platelets are considered warning signs. Plasma leakage, bleeding, and/or organ failure are considered severe dengue^{3,4,5}. Neurological complications can also occur in dengue infection⁶. Severe dengue includes central nervous system (CNS) impairment.

The neurological manifestations in dengue infection are caused mainly by DENV-2 and DENV-3. These serotypes are associated with cases of encephalitis, meningitis and myelitis^{7,8}. However, DENV-1 and DENV-4 are also identified in cases of encephalitis⁹. Although neurological manifestation is considered uncommon, recent studies showed that it is becoming more frequent in both DF and DHF^{2,10}. About 1-21% of individuals with dengue present neurological abnormalities^{7,11}. When evaluating the suspected cases of CNS infection, dengue was observed in 4-13%^{12,13}. The prevalence of DENV among viral infections in CNS is reported to be 5% and 6% in Vietnam^{14,15}, 15% in India¹⁶ and 20% in Thailand¹⁷. Our group also demonstrated that in a dengue endemic region (Rio de Janeiro, Brazil), DENV infection was the leading cause of viral acute encephalitis (47%) and was the etiologic agent of 10% of viral meningitis cases¹⁸. Therefore, it seems that neurological complications in dengue infection is becoming increasingly common and is not a rare observation. This fact can be related to the increased concern of the practitioners¹⁹. However, it is known that in a secondary dengue infection associated with a different serotype, an antibody dependent enhanced (ADE) mechanism can occur. In those cases, the heterotypic non-neutralizing antibodies interact with dengue virus, favoring the infection of host cell. As consequence, the viral replication is increased, as well the chances of dengue hemorrhagic fever. By hypothesis, the high replication rate may also contribute to the development of neurological disorders (Figure 1)²⁰.

The neuropathogenesis of DENV infection needs to be clarified. The CNS damage can be a result of four distinct mechanisms: (a) metabolic imbalance; (b) hemorrhagic disturbance (thrombocytopenia); (c) post-infectious autoimmune reaction; (d) CNS infection by dengue virus^{6,21,22}. The present narrative review summarizes the advances in the neuropathogenesis studies, in sense of to improve the understanding regarding the neuroinvasiveness, neurotropism and neurovirulence of DENV. Although these themes remain elusive, new knowledge has emerged in recent years.

NEUROINVASION: HOW DENV ACCESS THE NERVOUS SYSTEM

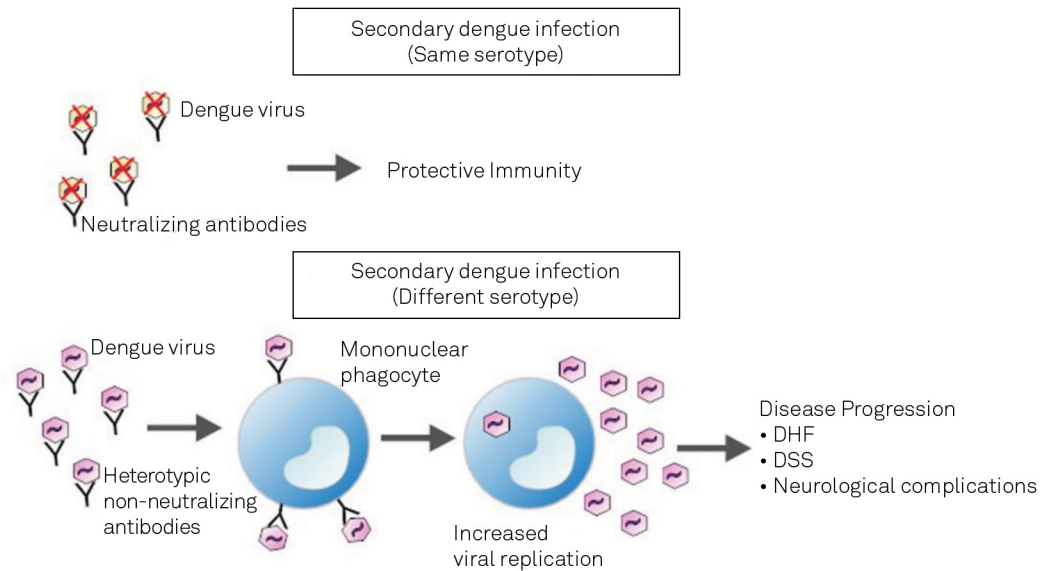
The ability of a microorganism to invade the nervous system is known as neuroinvasion. Hematological seems to be the most important route used by DENV to get into the nervous system. It is preceded by a viremia. The virus can disseminate as a free particle or inside of an infected cell (using a Trojan-horse mechanism of entry)²³. A study developed in

mice showed that DENV can breakdown the blood-brain barrier (BBB). The BBB is composed by endothelial cells of brain microvessels. During the infection, there is an over-expression of cytokines, that alter the permeability of the endothelium through the disturbance of the tight junctions^{23,24,25}. In fact, the break of BBB in dengue infection was associated with high levels of plasmatic metalloproteinase 9 (MMP-9)²⁴. MMP digests the basal lamina of neurovascular units, weakening the tight interactions between the endothelial cells and other elements of neurovascular units. Thus, this enzyme facilitates the entry of both free viral particle and infected leucocyte into cerebral tissue²⁴. Moreover, DENV-2-infected monocytes express monocyte chemoattractant protein-1 (MCP-1). *In vitro*, this protein is able to increase the permeability and disrupt tight junctions of human vascular endothelium cells. In fact, a high expression of MCP-1 was detected in DHF patient's plasma²⁶. Therefore, DENV is able to enter in the CNS. Another hypothesis is that dengue virus can access the nervous system, crossing the endothelial cells through transcytosis. This entry mechanism has been demonstrated in West Nile virus (WNV) infection²⁷. The ability of DENV to infect endothelial cells allows viral replication and may facilitate the subsequent entry into the brain parenchyma similar to what happens to other flavivirus infections^{28,29}. For hypothesis, it could be the same mechanism that the virus access the cerebrospinal fluid (CSF) cross the blood-CSF barrier. It contains fenestrated vascular endothelial cells on the choroid plexus, which may facilitate the neuroinvasion (Figure 2)³⁰. Moreover, the genetic characterization of DENV-4 obtained in a patient with encephalitis showed 99.99% of similarity between serum and CSF-derived viruses¹⁰.

It has been suggested the route of retrograde axonal transport as an alternative neuroinvasive mechanism. An et al.³¹ showed, using transmission electron microscopy, that DENV is able to penetrate and infect both CNS and peripheral nervous system neurons (motor neurons, axons and ependymal cells) in murine model. In the same study, the authors observed virion-containing vesicles that appeared to be fused to presynaptic membranes, reinforcing the ability of DENV to use the axonal transport²⁹. Based on this study, the axonal transport could also be used for virus dissemination throughout the nervous system. A negative serum with positive CSF (both collected in same day) DENV detected by polymerase chain reaction (PCR) reinforced this hypothesis⁷. Although, the presence of virus in CNS may be only the consequence of a passive crossing through the blood-brain and/or blood-CSF barriers. The virus was past in peripheral blood, before crossing the barriers into the brain. Therefore it could not be found in blood anymore.

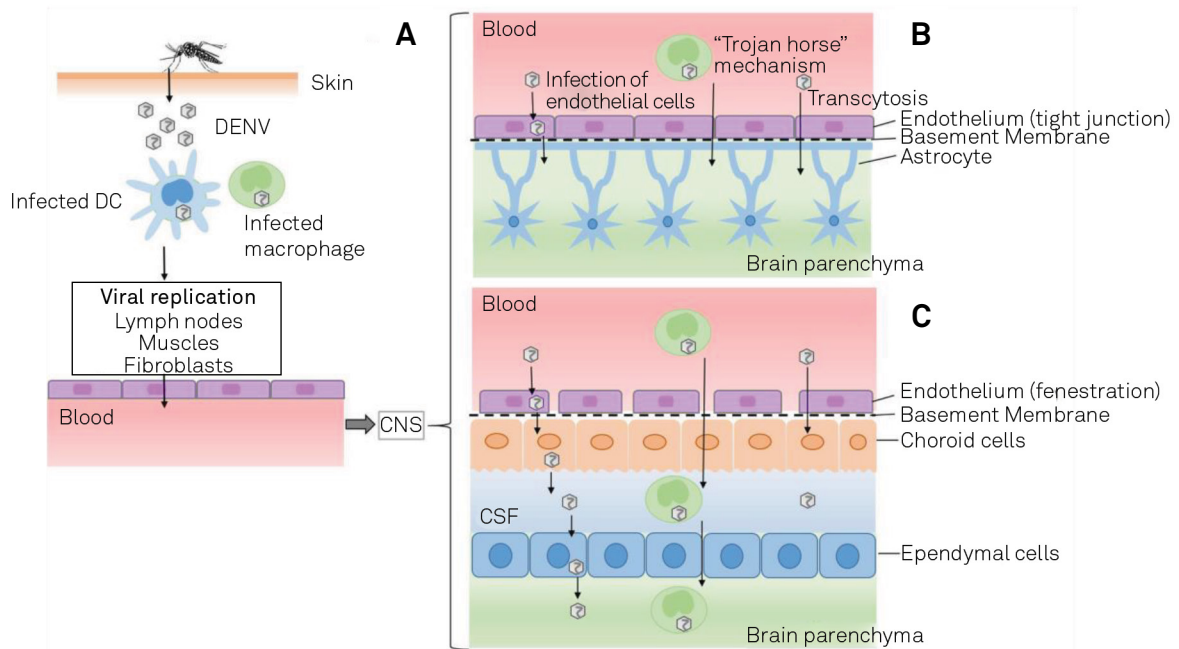
DENV NEUROTROPISM

There are several evidences that dengue virus is able to infect and replicate in neural cells. This ability is called neurotropism. Viral antigens were detected by immunohistochemistry



DENV: dengue virus; DHF: dengue severe hemorrhagic syndrome; DSS: dengue severe shock syndrome.

Figure 1. Antibody-dependent enhancement (ADE) in dengue virus infection.



DENV: dengue virus; CNS: central nervous system; CSF: cerebrospinal fluid.

Figure 2. Neuroinvasiveness of DENV in central nervous system. **A.** Viral invasion cross the skin after mosquito bite. It is followed by viral replication in lymph nodes, muscles and fibroblasts. The free particles or especially the infected monocyte disseminate the virus from the blood (viremia) to the visceral organ, inclusive the nervous system. **B.** Blood-brain barrier invasion. **C.** Blood-CSF barrier invasion.

in human cerebral tissue^{9,32,33,34,35}. DENV RNA was also detected in brain tissue and in CSF of infected individuals^{7,9,34,35,36,37,38}. Human and animal neurons, astrocytes, microglia and Purkinje cells can be infected by DENV. Moreover, plexus choroid and endothelial cells were also infected in animal and human^{25,32,33}.

The proteins Hsp70 and Hsp90, seems to form a candidate receptor complex to DENV entry in both human monocytes

and neuroblastoma cells^{39,40}. Another possible DENV receptor in neuroblastoma cells is the Mr 65,000 protein⁴¹. Salazar and colleagues demonstrated that dengue virus can bind to different host cell proteins that are present in both white and grey matter³⁹. NS1 dengue antigen was also detected in brain and in CSF of infected individuals^{42,43}. In animal model, previous studies showed that at early stages of the infection, only

a few virions were present in the cytoplasm of ependymal cells. However, with the progression of the infection, virions were observed in the lumen of the rough endoplasmic reticulum (RER), as also as RER-derived vesicles and the Golgi region of infected neurons³¹. This data demonstrates the DENV neurotropism. Another evidence of CNS infection is the viral detection of DENV-2, -3 and -4 in the CSF in cases of encephalitis, meningitis and myelitis. Additionally, intrathecal synthesis of specific antibodies (calculated by antibody index) was observed in DENV infected patients with myelitis. Therefore, the antibody index for DENV may be used as a marker of myelitis associated with dengue, and it seems to be associated to the pathogenesis of spinal cord disease due to direct viral invasion⁴⁴.

DENV NEUROVIRULENCE

Neurovirulence can be defined as the ability of the virus to induce neurologic disease. It is important to note that neurotropism is not a synonym for neurovirulence. However, in some cases, they may be associated. In fact, DENV infection of neuron induced apoptotic cell death in mouse model^{45,46,47}. The apoptosis may be induced by a cellular stress caused by an accumulation of viral proteins in cell membrane. For example, the neuroadapted BR/90 strain presents some mutations that prevent the maturation of the E protein. So, it results in an abortive virus assembly with an increased accumulation of viral proteins in cell membranes, which, in turn, seems to deflagrate apoptosis⁴⁶. Moreover, the viral infection may trigger the host immune response, which can contribute to neurologic damage. In this context, nitric oxide synthase expression correlates with death in dengue infection in murine model.

In some cases, the virus does not cause neurological disorder, even when infecting the central nervous system. Indeed, in some neuropathological autopsy studies, dengue virus or antigen have been detected in brain tissue without histopathological features of inflammatory reaction^{6,9,48,49,50}.

In this context, Bordignon and colleagues², using a mice model, showed that infection with a neurovirulent strain of DENV (FGA/NA) resulted in a higher viral load and progeny in CNS than the infection of the non-neurovirulent strain (FGA/89). Although the FGA/89 can efficiently infect CNS cells, it does not produce neurological disorder neither death of infected animals. In contrast, the neuroadapted virus FGA/NA can replicate more efficiently in CNS, causing extensive inflammatory process characterized by encephalitis and leptomeningitis as observed in histopathological examination². The genetic analysis of these viral strains showed three amino acid substitutions: one in E (structural) protein, and two in NS3 (non-structural) protein.

Interestingly, the genomic sequencing during the neuroadaptation process revealed that the mutations were

concomitant with the appearance of signs of encephalitis in mice². Genetic analysis of others neuroadapted strains reveals mutations that mapped to the same viral domain. While mutations in the E protein could enhance the neuropathogenicity of dengue infections by changing the virus binding or entrance into neuronal cells, the mutations in NS3 protein may increase the replicative capacity of DENV, which could explain the higher viral load observed in FGA/NA infection. These data together suggest that both E and NS3 protein are associated with neurovirulence in dengue infection in mice. The identification of molecular signatures associated with neurovirulence of DENV is extremely important as they can serve as molecular markers of neurovirulence. Nevertheless, when evaluating natural human infections, those findings were not observed. Complete genome characterization of DENV-4 did not reveal any of the described mutations associated with neurological strains. The same study observed that the majority mutations were in non-structural proteins encoding genes¹⁰.

FROM MOUSE MODEL TO HUMAN INFECTION

Non-human primates are not suitable models for neurological dengue once they present only a transient viremia, with no clinical signs⁵¹. Murine model is the most commonly used. Nevertheless, wild type DENV strains are not able to infect nor to induce mice death. So, the researchers have to use immunocompromised animals and/or DENV has to be adapted to this new host. In this adaptation process, which consists of several *in vivo* or *in vitro* virus passages, different mutations occur. These mutations take place throughout the genome, but are primarily observed in viral glycoprotein E^{2,46,47,52,53,54,55}. In respect to the pathogenesis of neurological dengue, these mutations detected in neuroadapted virus, may cause significant differences between what occurs in human infections to what happens in animal models. Indeed, those mutations were not observed in neurovirulent humans DENV. Moreover, several studies showed that the neurovirulent mutants selected by serial intracerebral passage in mice exhibited significant attenuation for human infection. In the past, this technique was even used to obtain attenuated virus for vaccine development⁵³. Therefore, this fact may generate an important bias regarding those studies. Another important issue is that in the majority studies regarding neurovirulence in mouse model, the virus was inoculated through the intra cranial route⁵¹. As the immune response varies according to the penetration route this can also cause discrepancies between what really happens in humans and what is induced in mice.

In murine model, neuroinfection was observed only in immune or neurological immature mice²⁵. However, cases of neurological complications due to dengue infection are observed in adult patients without any sign of immune impairment.

In conclusion, neurological complications in dengue infections are becoming more frequent. Neuroinvasion is not always associated with neurological disease. The demonstration of viral tropism and invasion in human nervous system are well established. Although, the role of viral factors (neurovirulence) still need to be clarified in humans. In addition, the identification of brain damage and viral markers are extremely important to understand the dengue neuropathogenesis. They can also help, in the future, to the establishment of a prognosis, disease control and vaccine development. The

intrathecal synthesis of specific antibodies to dengue virus seems to be a good marker of neurovirulence. Genetic analysis, mainly regarding E and NS3 protein, searching for specific mutations, can also be used as a potential neurovirulence marker in dengue infection. The current knowledge concerning neuropathogenesis of dengue virus has been hampered by the lack of an appropriate animal model of disease as also as limited autopsy data from fatal human cases. Although the available evidence is still preliminary, it suggests a direction to follow in future studies.

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