The mechanism of neuroinvasive disease in West Nile virus infection

Abstract

West Nile virus is a member of the *flaviviridae* family. It was first discovered in 1937 when it was isolated from blood of a febrile Ugandan woman. Since 1999, the virus has spread rapidly throughout the western hemisphere. Infection with West Nile virus often results in a fever. However, infection might also result in travelling of West Nile virus into the central nervous system and the brain, causing neuroinvasive disease. Infection might cause meningitis, encephalitis, poliomyelitis, chorioretinitis and has a high lethality. No specific treatment is available for West Nile virus infection. The mechanism by which the virus enters the central nervous system and the brain is not fully unraveled. Disruption of blood-brain barrier is important in causing neuroinvasive disease as well as travel of the virus into axons that lead to the central nervous system. Several factors modulate entry into the brain. Signaling of the tumor necrosis factor alpha receptor has shown to be a predominant factor in facilitating transport across the blood brain barrier. The aim of this review is to discuss the mechanism underlying neuroinvasive disease in West Nile virus infection.

Introduction

The West Nile virus (WNV) is an arthropod borne *flavivirus*, which is a member of the *flaviviridae* family. The *flaviviridae* virus family includes West Nile dengue, yellow fever virus, Japanese encephalitis virus and tick-borne encephalitis virus[1,2,3]. Most patients develop a fever. Since the outbreak of WNV in the United States (USA) in 1999, WNV has been an important cause of encephalitis (West Nile encephalitis, WNE). In this review we will discuss the pathology of a West Nile virus infection and the symptoms that are caused when the virus enters the CNS. These symptoms being: meningitis, encephalitis, poliomyelitis and chorioretinitis. We will take an in depth look in the cellular and molecular mechanisms that are important in the transmission of the virus into the brain and the central nervous system (CNS). Concludingly, we will discuss the future prospects to developing a strategy for treating neuroinvasive disease in WNV infection, and point out what still needs to be done in unraveling the exact details by which WNV enters the CNS.

Background

West Nile virus was isolated for the first time from blood of a febrile woman in Uganda, and was originally associated as a cause of fever in infected patients and sporadically with encephalitis. In the summer of 1999, WNV appeared in the US for the first time. Before, WNV only caused epidemics in Europe, Asia and Africa. This outbreak was marked with an unusual high amount of dead birds in New York[3]. This was followed by an outbreak of WNV in the human population, causing 62 cases of encephalitis, of which 7 persons died[4]. The *Centers for Disease Control and Prevention*(CDC) state on their website that since 1999, all states in the USA except Hawaii, Alaska, and Oregon have reported human, bird, veterinary or mosquito WNV activity. 28,743 confirmed cases of West Nile virus human illness (11,712 resulting in neuroinvasive disease) were reported, from which 1126 persons have succumbed to the disease from 1999 through 2008. The year 2003 marked the biggest outbreak of

WNV in the USA, with 9862 confirmed cases of WNV. Other outbreaks of WNV include Romania in 1998, Russia in 1999, and Israel in 2000[5,6,7].

WNV is arthropod born, which means the virus is mostly transmitted through mosquito vectors. The most predominant mosquito species carrying WNV are *Cx pipiens* and *Cx quinquefaciatus*. Both are members of the *Culex* species[8]. Though, the vector that is believed to be the most predominant in the 2003 epidemic in the USA is the *Cx Tarsalis* mosquito.

WNV infection and symptoms of neuroinvasive disease

Neuroinvasive disease is the most severe form of disease in WNV infection. Although approximately 80% of the cases are asymptomatic, WNV infection can be lethal if a patient develops neuroinvasive disease. The predominant symptom in patients that suffer from symptomatic WNV infection is a fever, which is present in almost 20% of the cases. Neuroinvasive disease is present in around 1% (1 in 150) of the patients with an WNV infection[3]. The mechanism by which West Nile infects the human body and causes fever, or more severely, a form of neuroinvasive disease, is explored later on in this review.

West Nile fever

The fever is not neuroinvasive and mostly develops after an incubation period of 2-14 days. Most people show a complete recovery from the disease. Nevertheless, the elderly have a higher mortality rate compared to younger persons and are at risk[1]. The fever is characterized as a flu-like illness with fever, myalgia, headache and occasionally gastrointestinal disturbance, including nausea and vomiting[9]. In some cases, a transient rash is presented, which is predominantly visible on the torso and extremities of a patient. The rash is more frequently shown in West Nile fever (WNF) compared with the neuroinvasive diseases in West Nile infection. In some persons, the rash lasts less than 24 hours, and is more frequent in younger persons than among the elderly[10]. The underlying mechanisms behind the differences in rash presentation remain unknown. Before WNV enters the CNS or the brain and causes neuroinvasive symptoms, patients usually develop a fever. The neuroinvasive symptoms that might follow WNF are discussed subsequently.

West Nile meningitis

Meningitis is characterized by meningeal inflammation, which is marked by physical signs such as nuchal rigidity (rigidity in neck muscle movement), kernig sign (the inability to completely extent the leg at the knee joint) and brudzinski's sign (bending the neck causes simultaneous bending of the hip and knees of the patient)[3]. Other clinical signs include an abrupt onset of fever, photophobia and phonophobia[1]. Additionally, West Nile meningitis (WNM) can also be accompanied with an augmented or decreased body temperature and increased leukocyte count in the cerebrospinal fluid (pleocytosis, 226 leukocytes/mm^3) or in the periphery (>10,000/mm^3).

Most cases of WNM have a favorable outcome, though hospitalization is needed in cases where patients are dehydrated by the associated vomiting and diarrhea[11]. Also, some patients experience persisted headache, fatigue and myalgias after recovering from infection[12].

West Nile encephalitis

West Nile neuroinvasive disease leading to encephalitis is most commonly seen in the immunocomprimised and the elderly. Particularly organ transplant recipients which are undergoing immune modulating therapy are at risk for West Nile encephalitis (WNE)[1]. WNE is indicated by encephalopathy, which is marked by a self-limited confusional state. Patients show a decreased level of consciousness, lethargy or personality changes. Some patients also develop a coarse, postural tremor or spasms , which are predominantly observed in the upper extremities and facial muscles. Other physical signs include hypomimia, bradykinesia and postural instability[13]. Occasionally patients with WNE develop a seizure. The encephalopathy varies in severity, between a 24 hour period of a self-limited confusional state through coma and death[12]. In addition, WNE can be accompanied with the same increased leukocyte count in the cerebrospinal fluid seen in WNM and the periphery with an acute inflammation in the CNS[3].

WNE generally has a less favorable outcome than WNM. The mortality rate ranges between 10% and 20% in normal individuals, while it is higher in the immunocomprimised and the elderly. Patients with WNE can show persistence in neurological dysfunction. This includes movement disorders, headaches, fatigues and cognitive complaints[1]. This may result in trouble with daily activities which often makes patients with persistent neurologic dysfunction require assistance with their functional and cognitive limitations[14]. Next to neurological features, patients also report neuropsychiatric symptoms. This includes depression, anxiety and apathy[12].

West Nile poliomyelitis

Although an infection with West Nile virus is often associated with movement disturbances and tremors, there are examples of more severe forms of weakness in the muscles in patients with an WNV infection. The virus may infect the lower motor neurons in the spinal cord which causes symptoms that resemble an infection with the Polio virus and the Guillain-Barré syndrome[15]. This particular syndrome in WNV infection is described as poliomyelitis. Generally, West Nile poliomyelitis (WNP) is marked by limb paralysis. Often asymmetric paralysis is shown, although symmetric paralysis is also observed in patients and is a more severe form of poliomyelitis. WNP often has a quick onset, mostly within the first 48 hours, the first symptoms of limb paralysis are observed. This acute onset of paralysis is also known as acute flaccid paralysis (or AFP syndrome) in West Nile infection[1]. Next to limb paralysis, WNP shows other symptoms with muscular involvement as well. Assessing data from patients with a West Nile infection who also had symptoms resembling Guillain-Barré syndrome showed that patients also developed respiratory failure, urinary bladder dysfunction, facial weakness and dysphagia. As much as 54% of the patients developed a respiratory failure which was marked by the requirement of artificial ventilation. 22% of the patients showed an urinary bladder dysfunction. Facial weakness and dysphagia was observed in 26% of the patients. In this group, infection of the cranial nerve was involved and seemed to be the underlying cause of the facial weakness and dysphagia. Just as in WNM and WNM, WNP shows pleocytosis. WNP marks an average of of 226 leukocytes/mm^3, and it is accompanied with a slightly elevated protein level in the CSF[16].

Recovery from WNP is marked by persistent weakness and disability and a high mortality rate. Most likely because the anterior horn cells which are infected in WNP are irreversibly damaged. Furthermore, during the late phase of the illness, muscular atrophy or diminished deep tendon

reflexes can be observed[2]. Patients with WNV infection that also present acute flaccid paralysis have the poorest prognosis. Patients that develop respiratory failure and a quadriplegic paralysis have the highest mortality rate. Though, recovery from WNP is often variable. The initial severity of the WNP infection is not necessarily a good prognosticator, and little is known about the long term prognosis for neurologic recovery. Although it is known that nearly all survivors have neurologic impairment to some extent[16].

Development of a post-polio-like syndrome after a West Nile infection with poliomyelitis is suspected in some patients is suspected and currently being assessed. Yet, little is known about this particular outcome of WNP[1].

West Nile chorioretinitis

Ophthalmologic involvement in WNV infection is not widely known. Though, 69% of the patients with West Nile fever or meningitis are known to have the chorioretinal lesions that mark West Nile chorioretinitis (WNC)[17]. These lesions usually manifest as granulomas and are most often clustered in the inferior fundus of the temporal and nasal regions. This particular distribution seems to be distinctive for WNV infection. Although it is known that many other infections and diseases within the ocular region can cause conditions in which chorioretinitis is observed. This includes syphilis, multifocal choroiditis, histoplasmosis, sarcoid, and tuberculosis. All of them have a distinguished pattern in their chorioretinal lesions which can be recognized and diagnosed as such[18]. Furthermore, WNV infected patients with chorioretinitis occasionally show ocular hemorrhages and many patients develop vitritis. Patients that develop chorioretinitis often complain about visual blurring and loss of vision. Also, floaters and flashes may be present in the vision of a patient[1].

Due to the fact that a significant percentage of the WNV infected patients develop chorioretinitis and its distinctive clinical picture, it is advised that medical examiners perform a detailed ophthalmoscopic examination. WNC often exists concomitantly with WNF and WNM, thus is able to aid in the diagnosis of a West Nile infection.

Most lesions in WNC disappear after the patient has recovered from the infection with WNV, although the chorioretinal symptoms can also be reduced by administering corticosteroids intraocularly[18]. Thus, WNC could also be observed as a clinical symptom in WNF and WNM. Though, WNC is still seen as a separate form of neuroinvasive disease in WNV infection, despite the fact that the West Nile virus has not yet been isolated from the intra-orbital region[1]. Little is known about the long term prognosis of WNC, since the chorioretinal symptoms of WNV have only been recognized recently. Up till now, a follow-up of patients with WNC showed that a few patients still had a worsened vision, while most patients had their vision return back to normal. A longer follow-up might reveal additional complications in patients with WNC, but this is yet to be determined[18].

Diagnosing a West Nile virus infection and therapy

When diagnosing a West Nile virus infection, there are a lot of other pathogens and associated diseases in the differential diagnosis. Neuroinvasive symptoms are not specifically a WNV infection symptom. These pathogens may not only cause the same symptoms, but also accumulate in the same areas in which WNV resides. For example, while WNV might be encountered in anterior horn

cells and the brain stem, the enterovirus 71 is also capable of residing in these areas. Other examples include the Polio virus, Nipah virus, Varicella-zoster virus, Herpes Simplex virus and rabies[19].

Table 1. Neurologic spectrum of WNE and WNE mimics

Brain related	Spinal cord related
Common	Common
Encephalitis	Flaccid paralysis[a] (anterior horn involvement)
Optic neuritis	
Chorioretinitis	
Tremors[b]	
Parkinson's disease[b]	
Myoclonus[b]	
Uncommon	
Cerebellar ataxia	
Paraparesis	
Quadriplegia	
Diaphragmatic paralysis	

[a]May also be present with Enterovirus 71, St Louis encephalitis, Japanese encephalitis, Murray Valley encephalitis, Powassan encephalitis, Louping ill, Coxsackie A7.
[b]May also be present with Japanese encephalitis, Nipah virus encephalitis, St Louis encephalitis, Enterovirus 71.

Infectious causes of acute encephalopathy	Noninfectious causes of acute encephalopathy
Herpes simplex virus-1	Acute toxic encephalopathy
Herpes simplex virus2	Acute metabolic encephalopathy
West Nile encephalitis	Hepatic encephalopathy
St Louis encephalitis	Systemic lupus erythematosus cerebritis
Venezuelan equine encephalitis	Granulomatous angitis
Western equine encephalitis	Central nervous system metastases
Eastern equine encephalitis	Cerebrovascular accident
LaCrosse encephalitis	Central nervous system hemorrhage
California encephalitis	
Japanese encephalitis	
Powassan virus	
Nipah virus	
Varicella-zoster virus	
Enterovirus 71	
Epstein-Barr virus	
Cytomegalovirus	
Human herpes virus-6	
Colorado tick fever	
Rabies	
Listeria monocytogenes	
Mycoplasma meningoencephalitis	
Legionella	

Source: Differential diagnosis of West Nile encephalitis. Cunha. 2004.

Table 1 and Table 2 show the neurologic spectrum of symptoms that are found in WNE, as well as possible mimicking infectious and noninfectious causes, as reviewed by Cunha, 2004. As can be observed, symptoms of WNE can be caused by other infectious pathogens, as well as a few noninfectious causes. Additionally, West Nile virus can mimic symptoms from other diseases. Examples include WNE mimicking CNS metastases, which seemed to be derived from small cell lung cancer, but was actually due to WNE[20]. Additionally, a case report has been described where WNE mimicked hepatic encephalopathy[21].

Although WNV infection often presents itself with features that are common for other pathogens, there are specific symptoms of WNV infection that can be distinguished from other infections, and aid in the diagnoses of a West Nile infection. The first thing to consider when patients present meningoencephalitis is to assess whether it is accompanied with flaccid paralysis. Also, as explained earlier, chorioretinitis may be a distinctive hallmark of WNV infection and aid in the diagnosis of WNV infection.

Due to the fact that a WNV infection can be mimicked by a lot of other agents, it is advised that a variety of tests is used to confirm a WNV infection. An example is shown in Table 3, which sums up the tests that are performed when WNE is suspected. As can be observed, a lot of tests can be used to confirm the diagnosis of WNE.

Table 3. Laboratory tests in WNE

Blood tests
Leukocyte count
Lymphocyte %
Platelet count
ESR, CRP
CPK levels
Ferritin levels
SGOT/SGPT
WNE IgM/IgG (ELISA) titers
CSF tests
Cell count/differential
Lactic acid
Radiologic tests
MRI
Brain
Spinal cord
Other tests
EEG
EMG (if weakness/paralysis)

ESR, erythrocyte sedimentation rate; CRP, c-reactive protein; CPK, creatinine phosphokinase; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; EEG, electroencephalogram; EMG, electromyogram.

Source: Differential diagnosis of West Nile encephalitis. Cunha. 2004

Serology

Testing serum for IgM antibodies is the most commonly used standard for diagnosis of WNV disease, since 75% of the WNV infected patients show an IgM antibody response to the infection on the fourth day. After seven days of infection, the percentage of patients showing an IgM response has risen to as much as 95%[19]. Therefore, measuring IgM levels is an excellent method to confirm an infection, and is usually performed by doing *enzyme linked immunosorbant assay* (ELISA). A high IgM titre is also observed in infections with other flaviviruses, which means there is a cross-reaction possibility. Therefore, it is advised that at least two serum samples are measured to confirm the diagnosis, by measuring a fourfold increase in IgM titres. It can also be confirmed by a *plaque reduction neutralization assay.*

Other ways of (directly) confirming a diagnosis of WNV infection may include *reverse transcriptase – polymerase chain reaction* (RT-PCR) of the virus in the serum. West Nile virus can also be demonstrated by immunohistochemically proving the presence of West Nile antigens[22]. These techniques are rarely used in the clinic. RT-PCR has a low sensitivity for West Nile virus, most likely because peak viremia occurs about 3-4 days before any symptoms are shown. Since RT-PCR is

generally performed in patients after the onset of symptoms, low viremia results in a poor sensitivity for the West Nile virus. A study in patients showed that RT-PCR was only able to confirm 14% of the patients with West Nile virus in serum.

Cerebrospinal fluid

West Nile virus infection is marked by pleocytosis, as described earlier. The pleocytosis has distinctive features in West Nile virus infection, including a prolonged predominance of polymorphonuclear cells, and the presence of plasma cell-like cells and mollaret-like cells. This pleocytosis is mostly accompanied with elevated protein levels (over 1g/l) and a high percentage of neutrophils in the CSF[19].

When neuroinvasive disease is suspected in a patient, samples are taken from the cerebrospinal fluid to confirm the diagnosis[2]. When performing an RT-PCR, sensitivity for WNV in the CSF is as high as 57%.

Radiology

Magnetic resonance imaging (MRI) can be used to measure abnormalities in the brain of WNV infected patients. Variable reports have been made about the outcome of an MRI study in patients. One study showed that within a group of 39 patients, only one person showed abnormalities on an MRI[23]. In contrast, other studies showed patients with lesions in the focal cerebral hemispheric white matter, pons, substantial nigra and abnormal signal intensity in the thalamus. Additionally, an abnormal signal intensity in the anterior horn cells corresponded to paralysis in WNV infected patients.[24,9,25] Therefore, if flaccid paralysis is present, an MRI of the spinal cord is needed in combination with an MRI of the brain.

Overall, the exact characteristics of a WNV infection on MRI need further examination. For this reason, the CDC has set up a WNV MRI registry. MRI can be sent to the registry, where they will be examined by experienced neuroradiologists, and sorted for statistical analysis in the hope that they will aid in composing a comprehensive picture on the imaging characteristics of WNV infection[26].

Therapy and prevention

There is no effective treatment for WNV infection. While several compounds have shown to suppress the virus, only a few of them show in vivo efficacy. Interferon α-2b has shown to inhibit the West Nile virus in vitro, and is currently in clinical trials[27]. CDC guidelines on treatment of WNV infection consists of general nursing care and supportive treatment including intravenous fluids and breathing aid.

Preventing spread of WNV is performed by limiting the spread of mosquitoes (vector control). The CDC recommends using insect repellent, staying indoors at dusk and dawn, have screens on the windows to keep mosquitoes out and getting rid of mosquito breeding sites, which is predominantly in stagnant water.

Vaccine development

At the moment, no vaccine is available to prevent a WNV infection. Current development of a West Nile vaccine is based on three strategies, as reviewed by Kramer et al, 2007. The first approach consists of synthesizing chimeric viruses with West Nile antigens. The yellow fever 17D strain have been transfected with genes from flaviviruses that produce the premembrane and envelope, which elicit an immune response. In addition, chimeric viruses have been made with West Nile virus and Dengue virus 2 and 4, which elicit an immune response as well. Chimeric viruses have shown to protect mice in vivo from a lethal dose of West Nile virus.

The second approach focuses on inactivated West Nile virus and West Nile particles. This includes recombinant viral particles, DNA that expresses viral antigens, formalin-inactivated West Nile virus, a recombinant canarypox virus vector and a DNA plasmid expressing the premembrane and envelope proteins of West Nile virus. Again, mice were protected in vivo from infection.

The third approach focuses on naturally less virulent West Nile virus subtypes. This includes the Kunjin virus, which is an attenuated West Nile virus subtype as well as WNV strain II. WNV strain I is a virulent WNV strain, and strain II has been shown to protect against a WNV strain I infection. Deleting genes from flaviviruses is a way to create artificially attenuated virus types, and has been shown to provide an immunogenic response. This includes Dengue virus, tick-borne encephalitis virus, and could be an alternative to developing an attenuated strain of West Nile virus.

Pathology and immune response in West Nile virus infection

When the West Nile virus enters the body (usually after a mosquito bite) it usually spreads to lymph nodes and the spleen, where viremia peaks after 2-4 days[1]. WNV is able to spread to the lymph nodes by infecting dendritic cells that migrate from the skin to the regional lymph nodes[28]. In response to infection, the dendritic cells often secrete various cytokines, including tumor necrosis factor alpha (TNF-α) and macrophage migration inhibitory factor (MIF)[29]. In addition, an interferon-dependent response by interferon (IFN) type I (α or β) or II (γ) have shown to inhibit replication of WNV during infection and plays a predominant role in viral clearance[30]. Cytokine secretion may modulate the permeability of the blood brain barrier (BBB) and thus modulate the spread of WNV into the CNS. The possible mechanism of the entry of WNV into the CNS is discussed later on in this review.

Risk factors modulating infection and pathology

As explained earlier, the elderly and the immunocomprimised have higher risk of acquiring a WNV infection and neuroinvasive disease. In addition, several other risk factors have been identified. A history of hypertension and cardiovascular disease are found to be an important risk factor for severe WNV infection. Additionally, chronic renal disease and infection with the Hepatitis C virus also increase risk of WNV infection[31,32].

Innate immune response

Sensing WNV in the body by the innate immune system is accomplished by recognizing the virus by Toll-like receptors (TLRs). The TLR family consists of a set of receptors that are especially important in initiating the innate immunity, by recognizing pathogen-associated molecular patterns (PAMPs). Activation of these receptors results in a downstream activation of transcription factors and subsequently an immune response is built up against the virus.

The production of IFN in WNV infection is influenced by TLR3 as well as TLR7. In addition to the TLRs, retinoic-acid-inducible gene I (RIG-I) and (to a lesser extent) melanoma-differentiation-associated gene 5 (MDA5) are involved in the recognition of West Nile virus. These pattern recognition receptors (PRRs) are able to sense WNV RNA and in response activate transcription factors that induce the expression of IFN[30]. These interferon regulatory factors (IRFs) have shown to protect against lethal WNV infection. The role of TLR3 in West Nile infection remains controversial. Although TLR3 recognizes viral RNA and influences downstream activation of IRFs and the production of IFN, mice lacking TLR3 show little change in viral load and IFN production. This shows TLR3 is not essential in activating an IFN response and effective clearance of the virus[43]

Mice lacking IRF-3 are significantly more vulnerable to WNV infection and have a higher virus titer. Interestingly, IFN- α and β production is only slightly attenuated.[33] Production of INF is hypothesized to be redundant by many other signals and thus not diminished when IRF-3 is absent. IRF-7 levels were found to be significantly higher in IRF-$3^{-/-}$ macrophages in mice and could be responsible for expression of IFN. Interestingly, cortical neurons susceptible for WNV infection show decreased levels of IFN type I when IRF-3 was absent. In addition, other cell types have shown to varying responses on IFN production when IRF-3 was absent. This shows that a cell specific IRF-3 response could be modulating WNV infection and indicates that IFN production is influenced by IRF-3-dependent and independent mechanisms [34].

In contrast to IRF-$3^{-/-}$, loss of IRF-7 results in a decrease of IFN type I production. IRF-$7^{-/-}$ results in increased WNV virus replication and lethality. This indicates that IFN production is influenced in an IRF-7-dependent way. Interestingly, IRF-7 is essential for the production of IFN- α, while IFN- β production is only slightly influenced by IRF-7[34]. Concluding, it can be said that protection from WNV infection requires an intact IFN type I response, and can be activated by various pathways.

The mechanism by which IFN affects antiviral pathways is to activate interferon stimulated genes (ISGs). ISGs have shown to inhibit viral translation and replication[30]. IRF-3 is also able to regulate the activation of certain ISGs. Macrophages express ISG54 and ISG56 in an IRF-3-dependent manner[35].

Adaptive immune response

Many components of the immune system are involved in the response of the body against WNV infection. In addition to production of INF, the immunological response in the periphery consists predominantly of a humoral response. An early accumulation of IgM and IgG antibodies in serum is necessary for survival of the host. Mice lacking an anti-WNV IgM and IgG response mostly succumb to lethal encephalitis after an infection with the virus[30]. Most of these antibodies recognize a

structural protein (E protein) on WNV, and a small portion recognizes the pre-membrane or membrane protein of WNV. CD8+ T-cells have also been observed in clearing the virus from the periphery by an antigen-restricted cytoxic response and secreting inflammatory cytokines. This suggests that although the humoral response is predominant, a cytotoxic T-cell (CTL) response is needed to fully clear the virus from the body[30].

In contrast to the peripheral immune response, clearance of WNV in the CNS requires a T-cell response. The exact effects of this cytotoxic response and its contribution to WNV infection outcome and pathology remain unclear. Though, it has been noted that the presence of CD8+ T-cells in the CNS contributes to clearance of the virus and thus preserve the brain from damage. The CTL response is usually initiated five days post-infection. A CTL response is also associated with increased pathogenicity, due to the killing of WNV infected neurons by T-cells. Nevertheless, the CTL response is associated with improved recovery from neuroinvasive symptoms[36].

Chemotaxis and leukocyte trafficking

WNV has several targets in the CNS. The virus may infect cortical, midbrain, cerebellar and spinal cord neurons, leading to various symptoms that have been described earlier in this review. In response to infection of neurons in the CNS, various chemoattractants and chemokines are released by neuronal cells to promote leukocyte trafficking into the CNS (predominantly CD8+ T-cells). Leukocyte trafficking is essential to develop an adequate immune response to WNV after it has entered the CNS and the brain. The influence of chemoattractants and chemokines on leukocyte trafficking is shown in Figure 1 and is discussed subsequently.

Expression of the chemoattractant CXCL10 by virus infected neurons promotes entry of CD8+ T-cells expressing the CXCL10 receptor CXCR3[37]. In much the same way, the chemokine CCL3-5 which binds to CCR5 promotes leukocyte trafficking into the CNS. CXCL12 has also shown to influence leukocyte trafficking. Polarized expression of CXCL12 and pharmalogical antagonism of CXCL12 has shown to increase leukocyte trafficking into the CNS parenchyma[38]. Though, the exact mechanism by which CXCL12 is expressed and regulated is poorly understood[30].

Neurons of the CNS have been commonly observed as bystanders during viral infection. Clearly, by promoting leukocyte trafficking into the CNS by various mechanisms, the neurons play an important role in inducing the CTL response in the CNS during WNV infection.

TLR7 has also shown to play an important role in leukocyte trafficking. TLR7 recognizes viral RNA in a myeloid factor 88 (Myd88) dependent way. Loss of TLR7 or Myd88 in results in decreased trafficking of $CD11b^+$ and $CD45B^+$ immune cells towards infected neurons[39]. TLR7 signaling results in the production of IL-23 in macrophages, which is essential for leukocyte infiltration into the CNS.

Lastly, TNF-α also regulates steps that induces specific trafficking of CD8+ T-cells and macrophages into the CNS, which are involved in viral clearance[40].

Mechanism of entry into the CNS by West Nile virus

The exact mechanism by which WNV enters the CNS and the brain remains unclear. Though, various components of the immune system have been found to be involved in modulating entry of the virus. Two distinct ways of entering the CNS can be noted in WNV infection: axonal transport and homogenous transport. As explained before, disruption of BBB integrity by MIF and TNF-α seems to play an important role in providing WNV a hematogenous route into the brain. Figure 1 shows the proposed mechanism by which the spread of WNV infection into the brain is promoted by MIF and TNF-α, and is accompanied with increased leukocyte trafficking across the BBB, which may be infected with WNV. Additionally, the ways in which WNV can disseminate into the CNS by means of axonal transport are shown.

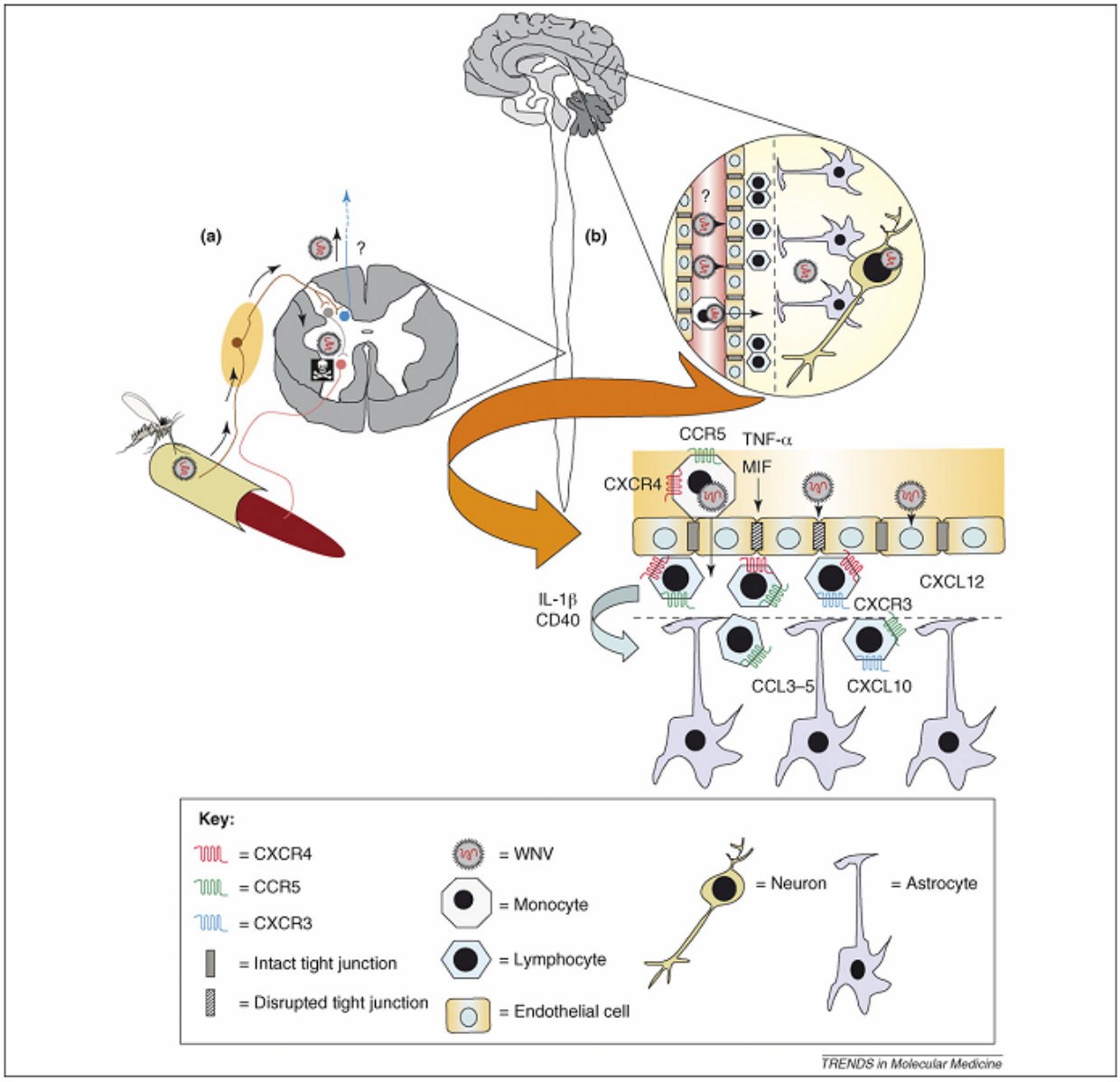

Figure 1. Mechanisms of WNV neuroinvasion and induction of leukocyte CNS entry. WNV might enter the CNS via axonal retrograde transport along peripheral neurons into the spinal cord (a) or hematogenously across the BBB (b). Spinal cord entry is believed to result in interneuronal spread to motor neuron cell bodies within the anterior root horns of the spinal cord, which might lead to flaccid paralysis. It is unclear whether retrograde transport continues along axons within dorsal spinal columns, allowing the virus to enter the brain. The routes of virus entry across the BBB might include intracellular transport within macrophages during physiologic turnover, intraendothelial spread during cytokine-mediated (TNF-α, MIF) BBB disruption or direct endothelial cell infection. Macrophages and lymphocytes that enter perivascular spaces are retained via CXCR4 binding CXCL12. Lymphocyte egress from perivascular spaces requires IL-1β and CD40. Virus-mediated upregulation of CXCL10 within neurons recruits virus-specific $CD8^+$ lymphocytes via CXCR3 activation, especially within the cerebellum. CNS upregulation of CCL3–5 leads to recruitment of CCR5-expressing leukocytes.

Source: Immunological headgear: antiviral immune responses protect against neuroinvasive West Nile virus. Klein et al. 2008

TLR3 mediates production of TNF-α. Loss of TLR-3 results in a decreased production of TNF-α and decreased TNF-α receptor 1 signaling (TNF-R1), which in turn resulted to a diminished compromise of the BBB[41]. Loss of TLR3 results in reduced infection, inflammation and neuropathology in mouse brains. Viral load was found to be higher in the periphery compared to wild type mice. This suggests that TLR3 plays a role in the recognition of WNV and initiation of the innate immune response, and additionally provides a way for WNV to disseminate into the CNS by leakiness of the BBB. Research in TNF-R1$^{-/-}$ mice also showed decreased viral load in the brain[39], although TNF-α plays an important role in the migration of leukocytes into the brain, and subsequently inducing viral clearance. This indicates that although there is a diminished migration by decreased TNF-R1 signaling, entry of WNV into the brain is even more impaired.

WNV itself plays a role as well in modulating BBB permeability and the host immune response. WNV produces nonstructural protein 1, which inhibits TLR3 signaling by preventing downstream activation of IRF-3 and the TNF-β promoter[42]. Though, downstream TLR3 mediated production of TNF- α and its receptor signaling is not inhibited. Therefore, WNV is able to inhibit the establishment of an antiviral state, but does not subsequently decrease BBB permeability by decreasing TNF-R1 signaling.

The exact role of TLR3 on BBB permeability remains controversial. Additional research of the loss of TLR3 in mice showed no altered TNF-α production or BBB permeability[43].

WNV may also enter the CNS by invading distal axons and disseminate into the CNS by axonal transport in retrograde and anterograde direction, as shown in Figure 1. WNV is able to travel towards the spinal cord and induce flaccid paralysis. The speed by which WNV is transported through the axons correlates with the onset of symptoms in West Nile neuroinvasive disease. Additionally, the ways in which WNV can spread into the CNS correlates with pathology of infection. Blocking certain neuronal routes with antibodies prevents paralysis in the corresponding limb in WNP[44]. WNV is also able to enter the CNS by movement across the olfactory mucosa by uptake of aerosols containing WNV[45].

Conclusion and future prospects

Although several factors have been found to play a role in neuroinvasive disease in West Nile virus, the exact mechanism remains unknown. WNV has various ways to cross the BBB, as can be observed in Figure 1. Whether WNV passes the BBB by infection of migrating leukocytes, infection of endothelial cells or passage through endothelial cells due to BBB disruption (or perhaps even a combination of factors) is unclear. Additionally, the innate and adaptive immune response associated with WNV infection may also modulate neuroinvasive disease. Peripheral clearance of WNV may contribute to preventing WNV to enter the CNS by disabling them to travel up to and infecting distal axons, by releasing neutralizing antibodies. In contrast, the immune response may also facilitate entry of WNV into the CNS by causing BBB disruption. The immune response consists of a cascade of reactions, and involves various cytokines, chemokines and white blood cells. Although certain factors have been found to modulate neuroinvasive disease, the exact characteristics of the immune response in the human body on causing or preventing WNV neuroinvasive disease is still unclear.

More research is needed to find the balance between clearing the virus and disruption of the BBB accompanied with neuropathology due to a CTL response.

Identifying the inflammatory cascade that causes neuropathology might give rise to novel targets in preventing neuronal damage. Since interferon type I is the most predominant factor in the immune response, it seems the most promising component in WNV therapy. This is backed up by studies performed in mice which were described earlier in this review.

Additionally, discovering the exact mechanisms by which flaviviruses induce infection and escape the response of the body might provide new targets for preventing WNV infection by developing a prophylaxis. As explained before, a WNV vaccine is currently in development and clinical trials are taking place. More knowledge about flavivirus pathology and virology might provide tactics to inhibit the possibility of WNV entering the CNS.

Other ways to prevent WNV infection might include a better understanding of the transmission cycle, and ways to prevent the spread of mosquitoes and infection in birds. Currently, the CDC has a set of guidelines to prevent mosquito breeding, which has been described earlier.

Symptoms of neuroinvasive disease in WNV infection are severe, and mortality is high. Because the exact mechanisms are yet to be unraveled, and no effective treatment is available, research regarding these subjects will probably keep going for many more years.

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