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Introduction to West Nile Virus

Shazeed-Ul Karim,

Fengwei Bai

Department of Cell and Molecular Biology, Center for Molecular and Cellular Biosciences, The University of Southern Mississippi, Hattiesburg, MS, USA

Abstract

West Nile virus (WNV) is a mosquito-borne, single-stranded, positive-sense RNA virus belonging to the *Flaviviridae* family. After WNV gains entry through an infected mosquito bite, it replicates in a variety of human cell types and produces a viremia. Although the majority of infected individuals remain asymptomatic, the manifested symptoms in some people range from a mild fever to severe neurological disorder with high morbidity and mortality. In addition, many who recover from WNV neuroinvasive infection present with long-term deficits, including weakness, fatigue, and cognitive problems. Since entering the USA in 1999, WNV has become the most common mosquito-borne virus in North America. Despite the intensive research over 20 years, there are still no approved vaccines or specific treatments for humans, and it remains an urgent need to understand the pathogenesis of WNV and develop specific therapeutics and vaccines.

Keywords

West Nile virus; Viral proteins; Transmission; Pathogenesis

1 Viral Genome and Proteins

WNV particle is spherical in shape with approximately 50 nm in diameter. It has an icosahedral nucleocapsid encircled by a lipid envelope [1]. The virus has an approximately 11 kb-long, single-stranded, positive-sense RNA genome containing a single open reading frame (ORF) flanked by untranslated regions (UTRs) at both ends, which extend to 96 nucleotides at the 5′ end and 631 nucleotides at the 3′ end in the epidemic strain WNV NY99 [2]. Like other flaviviruses, the WNV genome is 5′ capped with no polyadenylation tail at the 3′ end. The UTRs of the viral RNA genome assist in replication, transcription, translation, and packaging [3–5]. The viral RNA is translated as a polyprotein, which is cleaved by cellular and viral proteases into three structural proteins (capsid, envelope, and pre-membrane) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [2].

1.1 Structural Proteins

WNV capsid (C) protein encapsulates viral RNA to construct a nucleocapsid. The N- and C-terminals of the C protein have been reported to aid in RNA folding during viral replication, while the middle section of the C protein has been reported to involve direct interaction with viral RNA [6]. The envelope (E) protein is a transmembrane protein, and

its ectodomain contains three structural domains (DI, DII, and DIII). DI is comprised of a single glycosylation motif, DII contains an internal fusion loop, and DIII is an immunoglobulin (Ig)-like domain, which plays a crucial role in binding to cellular receptors and entry of the virus into cells [6, 7]. The pre-membrane/membrane (prM/M) protein is a small, glycosylated protein, which is essential in the formation and egression of progeny WNV particles. PrM protein, along with E protein, facilitates the formation of immature virions and inhibits a premature fusion during virus egress. Viral particles with immature prM are noninfectious until prM cleaves to M to stimulate the membrane-fusion activities of the virus [7].

1.2 Nonstructural Proteins

NS1 is a glycoprotein, which is found intracellularly, on the infected cell surface, or secreted by infected cells in the blood. The intracellular NS1 mediates viral replication and assembly, whereas the secreted NS1 regulates viral immune responses in the host [7, 8]. NS1 can bind to the regulatory protein factor H inhibiting the complement activation. It also interacts with C4, downregulates the production of type I interferon (IFN), and deactivates the classical and lectin pathways of the complement system. NS1 of WNV has also been reported to stimulate hyperpermeability of human brain endothelial cells to cause neurological disorders and facilitate encephalitis [9].

Both NS2A and NS2B are small transmembrane proteins. NS2A is a hydrophobic protein associated with the membrane of the endoplasmic reticulum (ER). It has been reported to form the scaffold for the viral replication complex and plays an important role in replicating the WNV genome, inhibiting host immune responses, producing virus-induced membrane structures, and assembling virion [10–12]. NS2B has been suggested to couple with NS3 protease cleaving viral protein to facilitate NS3 proteolytic activity and contribute to the host cell apoptosis and neuropathogenesis [7, 13].

NS3 is the best-characterized multifunctional protein, which is highly conserved in all flaviviruses. NS3 couples with the cofactor NS2B to form the NS2B-NS3 protease that cleaves the viral polyprotein into the structural and nonstructural proteins [14]. NS3 is also involved in helicase, nucleoside triphosphatase, and RNA triphosphatase activities that are essential to viral genome replication. NS3 helicase segregates double-stranded (ds) RNA during viral replication and promotes WNV resistance to antiviral action of $2'$, $5'$–oligoadenylate synthetase 1b (Oas1b). In addition, the WNV NS3 helicase has been shown to inhibit type I IFN-mediated antiviral responses [7].

NS4A and NS4B are small hydrophobic proteins connected by a 2K peptide composed of 23 amino acid residues. Both proteins are associated with membrane rearrangement in infected host cells and inhibiting IFN responses during WNV infection [7]. NS4A and NS4B have been shown to develop the scaffold for viral genome replication, inhibition of host immune responses, production of virus-induced membrane structures, and virion assembly [10–12].

NS5 is the largest nonstructural protein encoded by the viral genome. It is composed of N-terminal methyltransferase (MTase) activity and C-terminal RNA-dependent RNA-polymerase (RdRp) activity. The NS5 MTase activity is responsible for viral genome $5'$

capping, which mimics eukaryotic mRNA allowing the use of the host protein synthesis components for viral gene translation [15–17]. Like many other RNA viruses, WNV NS5 RdRp lacks a proof-reading mechanism; thus, the viral populations display a variable level of sequence diversity that may facilitate the viral evolution in response to selective pressures [7]. Besides the functions in viral genome replication, NS5 has also been suggested to have a significant role in the viral pathogenesis by inhibiting type I IFN responses of the host [18].

In summary, the structural proteins assist in forming virion structure, attachment, and entry, whereas the nonstructural proteins are responsible for viral genome replication, polyprotein translation, virion assembly, maturation, egress, and regulation of host immune responses. The primary functions of the WNV proteins are summarized in Table 1.

2 Epidemiology

WNV was first isolated from a female patient in 1937 in Uganda [19]; however, the first indication that its infection led to meningitis and encephalitis in humans was in the outbreaks in Israel in 1957 and France in the early 1960s [19–21]. WNV came into the spotlight when several outbreaks were reported in some parts of North Africa, Europe, and Israel, with severe neurological diseases cases and death incidence in the 1990s [22–24]. In 1999, WNV gained its entry into North America in New York City, and within 3 years, it had spread rapidly to most of the parts of the US and the neighboring countries, including Canada, Mexico, the Caribbean, and Central America [20, 21, 25]. In 2012, the USA witnessed an epidemic of WNV with 5,674 confirmed cases, among which 286 people died and over 50% developed neuroinvasive diseases [26–28]. Since 1999, approximately 25,000 neurological complications and over 2,300 deaths associated with WNV infections have been recorded in the USA [21, 29]. In 2018, a massive outbreak of WNV happened throughout 15 countries in Europe, with around 2,000 confirmed cases [30]. WNV has now spread throughout the rest of the world except Antarctica, and it is now considered the most important causative agent of human viral encephalitis worldwide. Therefore, there is an unmet need to understand the pathogenesis of WNV and develop specific therapeutics and vaccines.

3 Transmission

WNV transmission in nature maintains in a cycle between mosquitos and various bird species. Different species of *Culex* mosquitoes are the main transmission vectors of WNV worldwide. *Culex tarsalis* and *C. pipiens* have been suggested to be the primary vectors in the western and eastern parts of the USA, respectively [31–33]. The American robin (*Turdus migratorius*) is the most important host for the maintenance and transmission of WNV in the USA [34]. After a mosquito acquires WNV from a blood meal, the virus infects the midgut epithelial cells and starts its replication. WNV then travels to the salivary glands of mosquitoes via circulating fluid. When this infected mosquito feeds on humans or other animals, WNV may be inoculated into the host skin. WNV replicates in a variety of cells in humans, such as neutrophils, macrophages, and keratinocytes and generates a viremia. The viral load in blood in an infected mammal usually is not high enough as in birds to be transmitted to another mosquito; therefore, humans and other animals are considered

as the dead-end hosts in the WNV transmission cycle [35]. Besides mosquito bites, the transmission of WNV is also possible through blood transfusion, organ transplantation, breastfeeding, and laboratory-acquired infection [32–34].

4 Clinical Symptoms and Pathogenesis of WNV in Humans

WNV infection in humans is predominantly asymptomatic (80%), but it may result in a spectrum of diseases in about 20% of infected people. Among those symptomatic individuals, most may have a fever, malaise, headache, backache, myalgias, arthralgias, gastrointestinal symptoms (nausea, vomiting, or diarrhea), and maculopapular rash. About 1 in 150 people infected with WNV develop severe neuroinvasive diseases due to the viral infection in the central nervous system (CNS). Symptoms of neuroinvasive disease include high fever, severe headache, neck stiffness, confusion, stupor, tremors, seizures, muscle weakness or paralysis, and focal neurological deficits. About 10% of infected people develop severe neuroinvasive diseases such as flaccid paralysis, encephalitis, and meningitis die. Risk factors for encephalitis and death include advanced age, a history of cardiovascular or chronic diseases, and immunosuppression. Among those infected elderlies who survive WNV neuroinvasive diseases, as many as 50% may develop post-illness morbidity, such as fatigue, dizziness, difficulty concentrating, depression, anxiety, sleep disruption, recurrent headaches, and even autoimmune diseases, despite the clearance of infectious viruses from the body within a few weeks.

After WNV enters the human body through a mosquito bite, the virus replicates in keratinocytes and resident skin dendritic cells, i.e., Langerhans cells (LCs). Through blood and lymphoid circulations, WNV travels to and infects the peripheral organ such as the spleen, liver, and kidney. A low-level viremia is generated, which usually lasts for a few days. Infected LCs travel to the lymph nodes and spleen after being activated by WNV antigen, leading to T-cell activation [36]. Following WNV infection, dendritic cells produce a large amount of type I IFNs that may suppress the spread of the virus in the early phase of the infection. However, the signaling is decreased in DCs of the aged individuals infected with WNV, which could partially explain why WNV infection causes severe diseases in aged patients [37, 38]. Although the mechanisms of WNV entry into the CNS are not fully understood, multiple possible pathways have been suggested and verified in animal models. These pathways include flow through the tight junctions of the blood-brain barrier (BBB) from the blood circulation, direct infection of endothelial cells in the cerebral microvasculature, infection of olfactory neurons, “Trojan horse” transport via infected leukocytes, and/or direct axonal retrograde transport from peripheral neurons [9]. In the CNS, WNV can infect various types of cells, including neurons, astrocytes, and microglial cells, resulting in cell apoptosis or necrosis, inflammation of tissue and/or membrane of the brain, which may lead to encephalitis and meningitis.

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Table 1

Functions of WNV proteins

Proteins		Functions
Structural proteins	Capsid (C)	RNA folding, nucleocapsid construction, and regulation of cell apoptosis
	Envelope (E)	Receptor attachment and entry
	Pre-membrane/ membrane (prM/M)	Formation and maturation of viral particle
Nonstructural proteins	NS1	Viral replication and assembly, inhibition of the complement activation, and type I interferon production
	NS2A	Scaffold formation for viral replication, inhibition of host immune response, and virion assembly
	NS2B	Cofactor of NS2B-NS3 protease and polyprotein cleavage
	NS3	Proteolytic activity, helicase, IFN inhibition, and inhibition of antiviral response
	NS4A	Cofactor of NS3 helicase, membrane rearrangement, IFN inhibition, and regulation of immune response
	NS4B	Membrane rearrangement, IFN inhibition, and regulation of immune response
	NS5	Methyltransferase, RNA polymerase, IFN inhibition, and regulation of immune response