Vector-Borne Diseases & Treatment

Chapter 3

West Nile Virus: An Emerging and Reemerging Infectious Disease

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Abstract

West Nile Virus (WNV), a flavivirus of the *Flaviviridae* family, is maintained in nature in an enzootic transmission cycle between birds and ornithophilic mosquito vectors, although the virus rarely infects other vertebrates. WNV causes disease in horses and humans, which develops febrile illness, meningitis, encephalitis and flaccid paralysis. Until recently, its medical and veterinary health concern was relatively low; however, the number, and severity of outbreaks with neurological consequences in humans and horses have increased in European countries and the Mediterranean basin. Since its introduction in the America, the virus spread across the continent with worrisome consequences in bird mortality and a considerable number of outbreaks among humans and horses, which have developed in the largest epidemics of neuroinvasive WNV disease ever documented. Even great advances have been obtained lately regarding WNV infection, and although efficient equine vaccines are available, no specific treatments or vaccines available for human use. This review updates the most recent investigations of WNV particularly pathogenesis, transmission dynamics, host range, clinical symptoms, epidemiology, diagnosis, control, and prevention, and highlights some approaches that certainly require further research.

1. Introduction

West Nile virus (WNV) is a neuroinvasive human pathogen that is the causative agent of WNV in human and horses [1,2]. In 2000, the WNV spread out in 12 states and the Columbia District of U.S. WNV infect many avian and mosquito species throughout North America [3,4]. More than 2.5 million people affected by West Nile fever and encephalitis between 1999 to 2010. The outbreaks of West Nile virus occurred in U.S, Israel, Egypt, India, France, and South Africa. West Nile virus belongs to the family *Flaviviridae.* The family *Flaviviridae* comprises 3 genera: the flaviviruses, which contain WNV, dengue Virus (DENV), and yellow fever virus (YFV); the hepaciviruses, which contain hepatitis B and C viruses; and the pestiviruses, which causes encephalitis in hoofed mammals. Within the *Flavivirus* genus, which contains more than 70 viruses, viruses can be further classified into tick-borne and mosquito-borne viruses. The mosquito-borne viruses classified into the encephalitic clade, or the JE serocomplex, which contain WNV and Japanese Encephalitis Virus (JEV), and the nonencephalitic or hemorrhagic fever clade, which contain DENV and YFV, and there are 10 serologic/genetic complexes [5,6,7]. The global distribution of the mosquito-borne flaviviruses depends on the habitat of the mosquito vector. *Culex* mosquitoes transmitting encephalitic flaviviruses mostly in the Northern Hemisphere.

2. Structure of the Virus

WNV is an enveloped virus having a single-stranded, positive- sense RNA genome. The genome comprises of a single open reading frame of about 11 kb length. Both the 5' and 3' noncoding regions of the genome synthesize stem-loop structures that help in replication, transcription, translation, and packaging [8,9,10]. The West Nile RNA directly translated in to a single polypeptide and then cleaved by host and viral proteases. Followed by the formation of three structural (capsid, envelope, and premembrane) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins. Structural proteins are essential for entry of virus in to host cell and also aid in fusion encapsidation of the viral RNA during assembly **[Figure 1]**.

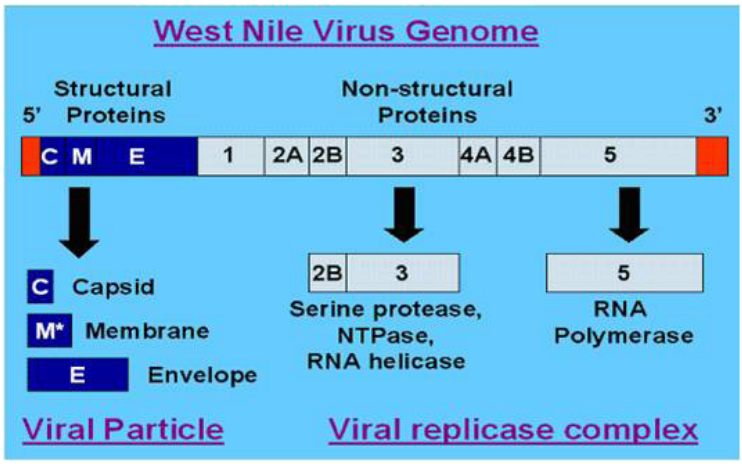

Figure 1: Schematic of genomic Organization of West Nile Virus .The author acknowledge the support from; http://cme.cwru.edu/brochures/2003/WESTNILE

The nonstructural proteins play important role and perform several functions. NS1 protein has "cellular" as well as secreted form and has highly immunogenic but do not play important role during encapsulation and packaging, but it plays significant role in replication of viral genome [11,12,13,14]. NS3 protein suggested to play important role in cleaving of viral polyproteins with the aid of the viral proteases and encodes enzyme activities. The NS5 protein is essential for viral replication because it has viral polymerase activity and encodes a methyltransferase. Other nonstructural proteins, including NS2A, NS2B, NS4A, and NS4B, have been shown to prevent several components of the innate immune system against viral infection [15,16,17]. West Nile virion is an icosahedral particle. The capsid protein associating with the viral genome to form the nucleocapsid and lipid bilayer is present outside of the nucleocapsid. A high ratio of capsid protein localizes to the nucleus, while viral assembly always occurs in the cytoplasm. Budding is takes place in the endoplasmic reticulum (ER) [18,19,20]. Although inside the nucleus functions of capsid are not fully known, recent researches suggests a role in gene regulation. During virus assembly, the envelope protein enclosed firmly in the lipid bilayer of the virus and display to the virion surface [21,22,23,24]. The envelope protein is necessary for binding to the receptor on the cell surface for viral entry. The prM protein is also known to enclosed firmly in the lipid bilayer. At the time of infection, the virus population comprises both mature and immature virion particles [25].

3. Life Cycle

WNV enters in to the host cell through receptor-mediated endocytosis process. Several molecules have been serves as receptors for West Nile virus, like DC-SIGN, mannose receptor, and several glycosaminoglycans etc. Endosome of the virus matures during internalization from the cell surface, with the pH decreases down from neutral to slightly acidic in the early endosome and having more acidic at the time of maturation in to the late endosome. Inside the late endosome, the envelope protein changes their conformation and then viral lipid membrane fuse with the endocytic membrane and viral RNA genome releases into the cell cytosol. After capsid disassociation, the viral genome is replicated and assembled. The viral polyprotein is translated and processed on intracellular membranes of cell organelles and expression of the 10 viral proteins occurs. The viral genome is replicated with the help of viral and cellular proteins [26].

Immature flavivirus particles also play significant role during infection. Thes immature flavivirus particles form during inefficient cleavage of the prM protein at the time of maturation and budding. Immature flavivirus particles were traditionally thought to be noninfectious, several reports have shown that immature WNV particles may be potentially immunogenic and infectious *in vitro* and *in vivo* when linked by antibodies against the E or prM protein. These antibody-linked immature virus particles penetrate the immune cells via the Fc receptor of the antibody resulting infection occurs [27] **[Figure 2]**.

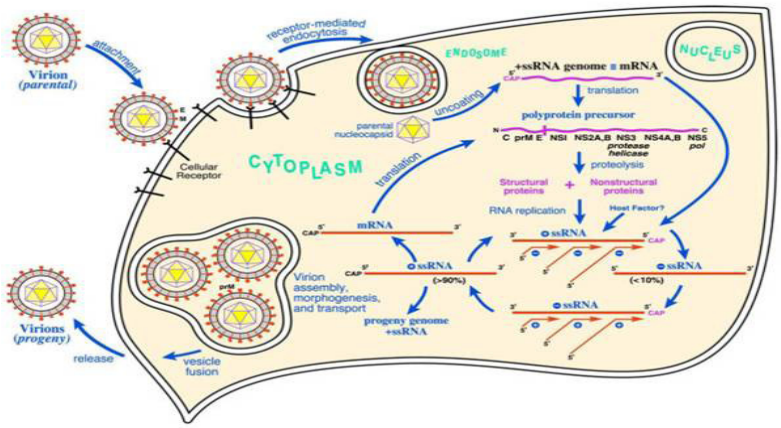

Figure 2: The West Nile virus life cycle. During viral entry, the E protein interacts with one or more cell surface receptor (s). It is not completely clear which cellular receptors are involved in WNV binding, however DC-SIGN, alphaVbeta3 integrin and laminin-binding protein have been reported as potential receptors. After binding to the cell, the virus is taken up via clathrin-mediated endocytosis and in the acidified endosome the E protein undergoes conformational changes resulting in fusion between the viral and cellular membranes. After the fusion event the positive-stranded RNA genome is released into the cytoplasm of the cell. The viral RNA is translated into a single polyprotein, which is proteolytically processed to yield three structural proteins (the envelope protein E; the membrane precursor protein prM; and the capsid protein C) and seven Non-Structural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). Whereas the cleavages at the junctions C-prM, prM-E, E-NS1, NS4A-NS4B, and likely also NS1-NS2A, are performed by the host signal peptidase located within the lumen of the ER, the remaining peptide bonds are cleaved by the virus encoded NS3 protease. Flaviviruses replication requires the viral protein NS5, which is an RNA-dependent RNA polymerase. An "antisense" negative strand RNA is produced by this enzyme, which then serves as a template for the synthesis of many new copies of the infectious positive strand RNA genome. The author acknowledge CDC to obtain this image.

4. Host Reservoirs

WNV is survive in nature in a cycle between mosquitoes and animal hosts. Some species like birds show symptoms of disease, and may die, while others become only infected do not show symptoms and act as carriers [28,29,30]. Although house sparrows and crows are play minor role in transmission. The American robin play important role in transmission of WNV in the United States [31,32]. Humans are considered as “dead-end” hosts for WNV, as the low level of viremia in mammals is usually not sufficient to be transmitted to mosquitoes, thereby ending the transmission cycle. The ability of mammals to serves as hosts *Aedes* mosquitoes, feed primarily on humans, become primary transmission vectors for WNV [33,34] **[Figure 3]**.

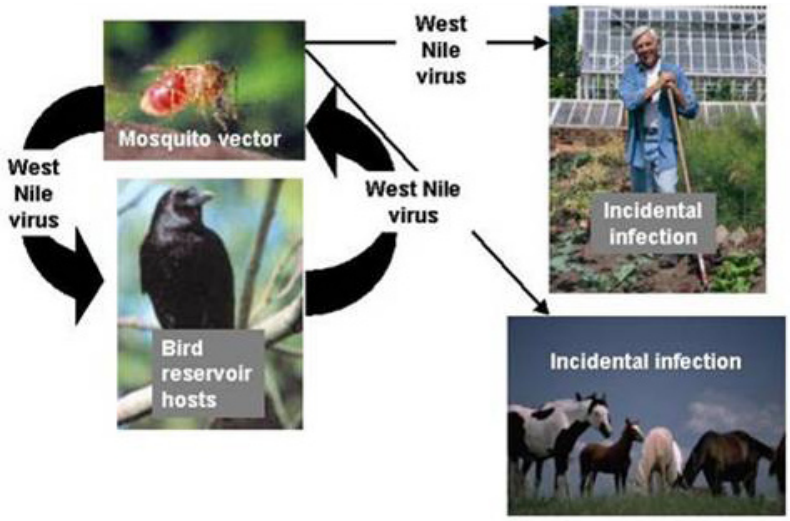

Figure 3: Transmission cycle of West Nile Virus The author also thankful to CDC to get above image

5. **Vector Response to Infection**

There have been many recent discoveries purposed at clarifying the transcriptomic and proteomic response to flavivirus infection in the mosquito vector. WNV demonstrate a consistent infection in mosquito cells *in vitro* and in live mosquitoes, there is growing evidence that the mosquito does show some immune responses against virus infection. Reports about the insect immune system come from experiments with *Drosophila melanogaster*, though recent examination of the mosquito immune responses is starting to expose related proteins and pathways [35,36,37]. The mosquito antiviral response is include two pathways: the innate immune pathway and the RNA interference (RNAi) pathway. The innate immune response is included of three signaling pathways: Toll, JAK-STAT, and IMD. The Toll and IMD pathways both ends in NFkB-mediated expression of antimicrobial peptides (AMPs) and IMD signaling has been shown to prevent RNA virus infection in *Drosophila* [38,39,40]. Not much is known about the role of mosquito AMPs in antiviral immunity. Their expression is induced by viral infection. Both Toll signaling and the JAK-STAT pathways have been show significant role in the inhibition of DENV infection in *Aedes aegypti* [52,53] and may also be significant at the time of infection of *Culex* with WNV. The RNAi pathway in mosquitoes is both activated by viral ds RNA and has been shown to be crucial for preventing alphavirus infection in *Aedes* and *Anopheles* [41,42,43]. The RNAi pathway is induced at the time of WNV infection in *Culex pipiens* [44]. Infection with DENV was also found to actively control mosquito immune responses *in vitro* [45]. Evidence for a transcriptomic approach of flavivirus infection was found at the time of a comprehensive study of *Aedes aegypti* infected with WNV, DENV, and YFV [46].

Genes play significant role in transcription and ion binding. There is upregulation and down regulation of genes takes place here and genes coding for proteases and cuticle proteins were to be up regulated at the time of infection with all three viruses. Serine proteases play significant role during viral propagation and blood digestion, although there have been several studies related their effect on flaviviral infection in the mosquito [47,48]. Another report of flaviviral infection in *Drosophila* recognized several insect host factors relevant at the time of dengue virus infection in the mosquito. In regarding to WNV infection, a recent transcriptomic analysis of *Culex quinquefasciatus* exposed that several genes takes part in metabolism and transport are upregulated at the time of infection [49,50]. The virus infect a variety of cell types and organs in the mosquito vector, and other host factors which play significant role in WNV infection of the mosquito that have yet to be reported.

West Nile (WNV) virus is mosquito-transmitted flavivirus that cause significant morbidity and mortality worldwide. Disease severity and pathogenesis of WNV infection in humans depend on many factors, including pre-existing immunity, strain virulence, host genetics and virus–host interactions [51,52,53]. Among the flavivirus-host interactions, viral evasion of type

I interferon (IFN)-mediated innate immunity has a critical role in modulating pathogenesis. DENV and WNV have evolved effective mechanisms to evade immune surveillance pathways that lead to IFN induction and to block signaling downstream of the IFN-α/β receptor. Molecular mechanisms of DENV and WNV antagonize the type I IFN response in human cells [54,55].

6. Molecular Classification

Classifications of WNV were depends on cross-neutralization reactions and showed that WNV is a family member of the Japanese encephalitis virus serocomplex. Recent studies on molecular phylogeny suggest this antigenic classification and expose the presence of up to eight different genetic lineages of WNV. Lineage 1 is further divided into three clades. Clade 1a consists of African, European and American isolates; clade 1b groups the Australian Kunjin Virus (KUNV), which has been reveal a subtype of WNV and clade 1c clusters isolates from India. Clade 1a reveal close genetic relationships between globally different areas which are suggested to be the result of WNV transmission *via* migratory birds WNV inside clade 1a can be further classified into various clusters. The only one endemic genotype has been identified in India (1c) and one in Australia (1b), suggests that WNV was spread into these areas only once, as well as it occurred in the American continent, where WNV was endemic in 1999 in the East Cost of the US [56].

The first North American WNV isolate was show relevancy to a strain isolated from a dead goose in Israel (lineage 1) North American WNV was originated from this epidemic in the year 1998 outbreak. However, recent studies suggest that the 1998 Israel epidemic was not the directly relevant of North American epidemics, but rather that both epidemics derived from the same (unknown) areas . Lineage 2 initially consists of WNV strains only identified in Africa and Madagascar, which have been speculated to be less neuroinvasive than those consists in lineage 1. Recent outbreaks occurs in Europe (Austria, Hungary and Greece) have been related to lineage 2 strains. Other lineages of WNV of unidentified human pathogenicity due to lineage 3 (Rabensburg isolate 97-103), isolated from *Culex pipiens* mosquitoes in the Czech Republic in 1997 and Lineage 4 (LEIVKrnd88-190), isolated from *Dermacentor marginatus* ticks in 1998 in Russia. It has been suggested that WNV Indian isolates that were classified as linage 1c forms a new cluster termed lineage 5. A new lineage of WNV (strain HU2925/06) that includes evolutionary branch with lineage 4 has been recently reported in Spain [57] **[Figure 4]**.

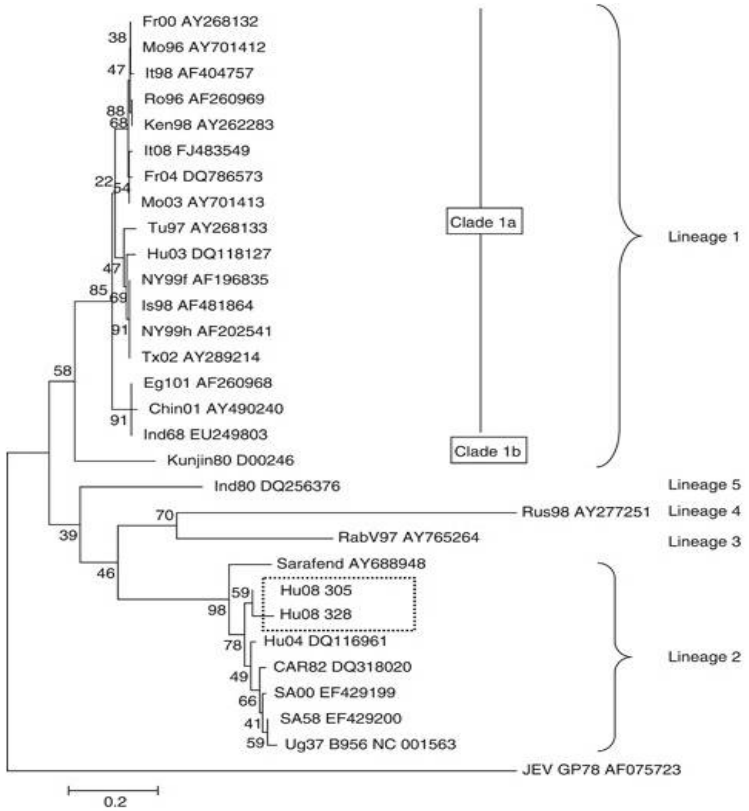

Figure 4: Phylogenetic tree of West Nile virus strains based on a 282nt fragment of the Envelope gene. The tree was constructed with the program MEGA (Molecular Evolutionary Genetic Analysis) by neighbor-joining. Bootstrap confidence level (1,000 replicates) and a confidence probability value based on the standard error test were calculated by MEGA. WNV strains are named according to the following rules: a set of letters corresponding to the place where the strain was isolated (Fr, France; Mo, Morocco; It, Italy; Ro, Romania; Ken, Kenya; Tu, Tunisia; Hu, Hungary; NY, New York; Is, Israel; Tx, Texas; Eg, Egypt; Chin, China; Ind, India; Rus, Russia; Rab, Rabensburg; CAR, Central African Republic; SA, South Africa, Ug, Uganda), 2 numbers for the isolation year (ex: 00 = 2000, 96 = 1996), and GenBank accession number. Sequences obtained from the 2 horse samples in Hungary 2008 are highlighted (rectangle). JEV, a close flavivirus, was used to root the phylogenetic tree. The author thanks to research gate for this image.

7. Molecular Epidemiology

The WNV outbreak occurred in North America in the late summer of 1999 in New York City due to this outbreak of mosquito borne encephalitis death occurred of humans, birds, and horses [58]. WNV transport throughout the United States and into Canada, Mexico, and the Caribbean **[Figure 5]**. From 1999 to 2016, 46,086 cases were reported to the CDC, with 2,017 (4%) deaths, and 21,574 reported cases of neuroinvasive disease, with 1,888 (9%) deaths [Figure 6]. As in most cases the virus is spread by the *Culex* mosquito vector. WNV transmission may occur through blood transfusion, organ transplantation, and laboratory-acquired infection has also been reported. The first reported Acute Encephalitis Syndrome (AES) outbreak in Kerala, India, occurred in Kuttanad region between January and February 1996, causing 105 cases and 31 deaths. In India, presence of West Nile antibodies in humans was first reported from Bombay (now Mumbai) by Banker in 1952. Smithburn et al confirmed the report by detecting the WNV neutralizing antibodies. During a post sero-epidemiological study, detected WNV neutralizing antibodies among humans at South Arcot district of Tamil Nadu. WNV has been isolated from sporadic cases of encephalitis and mosquitoes. Work postulated a hypothesis of a zoogeographical interface of Japanese encephalitis and West Nile virus. The hypothesis

proposed the intermingling distribution of JEV and WNV at the south Indian peninsular region [59].

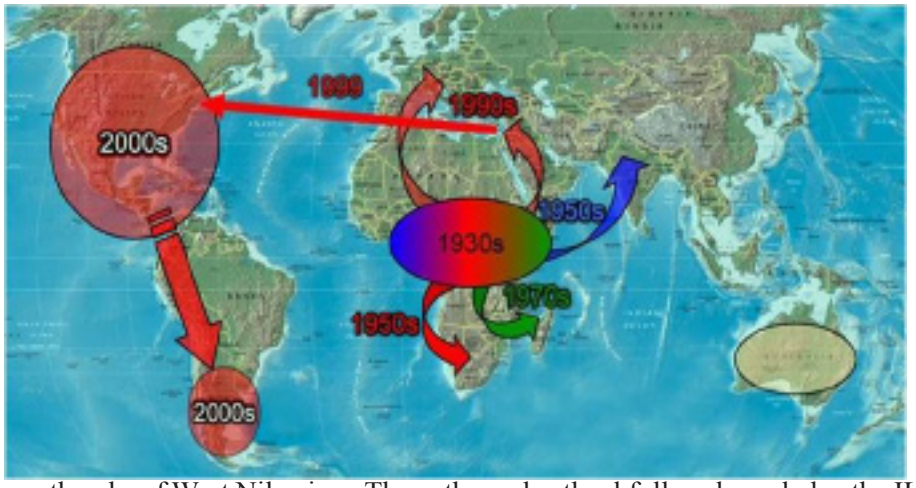

Figure 5: Worldwide outbreaks of West Nile virus. The authors also thankfully acknowledge the ILRI Clippings for this image.

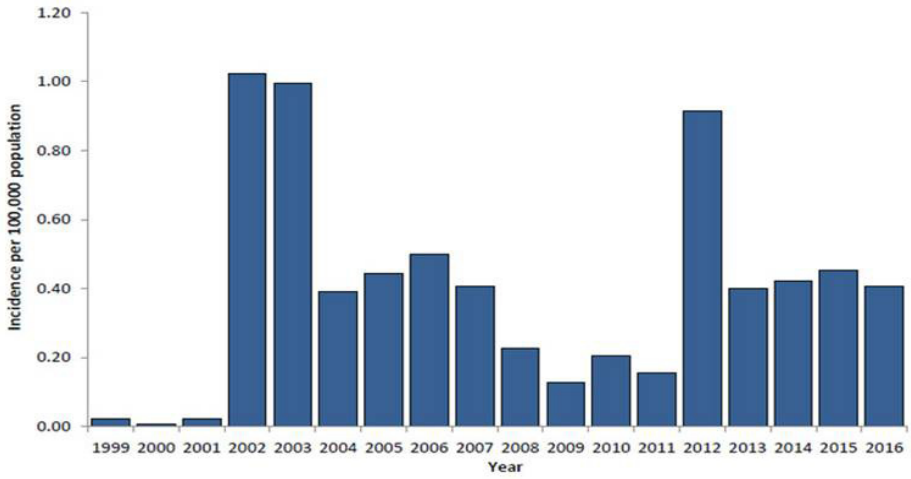

Figure 6: West Nile virus neuroinvasive disease incidence reported to CDC by year, 1999-2016. The author acknowledge the CDC to get above mentioned information

8. Clinical Features

Infections in humans are mainly subclinical, but reported infection demonstration may range from fever and CNS affected in encephalitis causes death. Encephalitis occurs only in a small subset of patients; concatenation to chronic neurological condition may induce acute paralysis after encephalitis. Individuals with neurological involvement that may result in death and may have high risk of mortality after acute illness. In about 75% of infections people have few or no symptoms. About 20% of people develop a fever, headache, vomiting, or a rash. In less than 1% of people, encephalitis or meningitis occurs, with associated neck stiffness, confusion, or seizures. Recovery may take weeks to months. The risk of death among those in whom the nervous system is affected is about 10%. Among people over 70 years of age, the case-severity rate ranges from 15% to 29% [60-62]. Higher mortality is also seen in infected infants and in immunocompromised patients. Risk factors for encephalitis are cardiovascular disease or chronic renal disease, hepatitis C virus infection, and immunosuppression. In some cases convalescent patients may have persistent or severe infection identified through molecular

based assays like PCR of the urine, which suggested ongoing viral replication in kidney tissues [63,64].

9. Pathogenesis

WNV is a neuroinvasive disease involve in progression of meningitis or encephalitis to poliomyelitis- like condition with acute flaccid paralysis that can also cause respiratory troubles. It is estimated that 10% cases are fatal [65,66]. Older age individuals are at high risk of infection but neuroinvasive diseases have been also reported in young people and children. Hypertension and diabetes are considered as potential risk factor for severe WND, Immunocompromised people easily infected by this disease. A fully functional immune (innate and adaptive) response (humoral and cellular) has been shown to be necessary to compete with WNV infection in animal models. It is well familiar that overall, humoral immune response has capable of control viral load while T-cell mediated response is necessary for clearance of the virus from the Central Nervous System (CNS). WNV is able to infect neurons of the CNS, brain, stem and spinal cord [67,68].

The pathogenesis of WNV infection is similar to that of other Flaviviruses. After primary inoculation, WNV is replicate in skin Langerhans dendritic cells before it travels to the lymph nodes and blood stream from where it transfers to the spleen and kidneys and, finally reaches to the CNS resulting in inflammation of the medulla, brain stem and spinal cord. The Viral entry mechanism in CNS is yet to be fully elucidated. The main mechanisms include: *via* leukocytes, direct entry across the brain barrier [69,70].

As several viruses, WNV has developed different mechanisms to block the action of immune system like it inhibit Interferon gamma (IFN) and, thus, to escape the host antiviral activity of IFN-stimulating genes. Different reports suggest that nonstructural proteins NS1, NS2A, NS4B and NS5 play important role to control IFN α/β signaling by several pathways [71,72].

10. Laboratory Diagnostics

Routine laboratory diagnosis of WNV infection is primarily based on serodiagnosis, followed by virus isolation and identification. Serologically, WNV infection can be inferred by immunoglobulin M (IgM) and immunoglobulin G (IgG) capture ELISA. Recently several investigators have reported PCR-based detection systems for the rapid diagnosis of WNV infection in clinical specimens that are negative for virus isolation, suggesting that nucleic acid-based assays hold great promise for the diagnosis of WNV infection. In addition, other PCR-based methods, like Reverse transcription loop-mediated isothermal gene amplification (RT-LAMP) assay, have been developed for the diagnosis of WNV RNA [73].

10.1. Immunological diagnosis

The detection of WNV infection is mainly based on clinical criteria and testing for antibody responses. The incubation period for WNV infection is about 2 to 14 days. The anti-WNV IgM mainly from Cerebrospinal Fluid (CSF), is used for detection. Cross-reactivity with related flaviviruses (Japanese encephalitis virus, St. Louis encephalitis virus, YFV, and DENV), if suspected, can be accessed through plaque neutralization assays (PRNT). Replication of WNV has been reported in human monocytes *in vitro* and with higher efficiency in polymorphonuclear leukocytes; this could cause transmission via blood transfusion [74,75]. Thus many rapid diagnostics have been developed for blood donor testing using Nucleic Acid Testing (NAT), an amplification-based transcription technique, which detects WNV-infected individuals before they become symptomatic [76,77]. RNA mostly became undetectable after 13.2 days, although it was rarely found to persist for 40 days. IgM and IgA antibodies decreases significantly, while the IgG level left elevated for 1 year after detection of viremia. Antibody to WNV NS5 persists *in vivo*, and thus NS5 antibody cannot be used to differentiate recent from old WNV infection [78,79].

10.2. Molecular diagnosis

Several methods for identification of viral RNA have been used for WNV surveillance. Generally Reverse Transcription Polymerase Chain Reaction (RT-PCR) technique, quantitative real-time RT-PCR and Isothermal gene amplification techniques like LAMP that is one-step, single tube, cost effective real-time reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay was developed for identifying the West Nile (WN) virus [80]. The RT-LAMP assay is a novel technique of nucleic acid amplification that is highly specific, sensitive, rapid and cost effective under isothermal conditions with a set of six specially designed primers that recognize eight distinct regions of the target. The complete process is very simple and rapid, and amplification can be occur in less than 1 h by incubating all of the reagents in a single tube with reverse transcriptase and *Bst* DNA polymerase at 63°C. *Bst* DNA polymerase has strand displacing activity. There are several formats for end point detection of gene amplification like agarose gel electrophoresis, real-time monitoring in an inexpensive turbidimeter, in practice, generally the visual inspection for amplification is performed through observation of color change following addition of SYBR green I dye, (a fluorescence ds intercalating dye) When the sensitivity of the RT-LAMP assay was compared to that of conventional RT-PCR, it was found that the RT-LAMP assay demonstrated 10-fold higher sensitivity compared to RT-PCR. All these assays have been extensively used in mosquito pools, animal and human samples [81,82].

11. Therapeutics and Vaccines

Advance therapeutic alternatives against WNV are mainly supportive; there are no FDA-approved vaccines or treatments available. RNA interference (RNAi) is a major pathway of antiviral defense in plants and insects. The pathway involves the processing of Double-Stranded RNA (dsRNA) into short 21–22 bp effector RNA molecules by the RNAse III domain-containing Dicer 2 enzyme. The fidelity of Dicer cleavage and downstream strand selection in the RNAi process is maintained by Dicer-associated proteins (TRBP and PACT in mammalian cells) These dsRNA serve as targeting moieties when loaded into a RISC complex to selectively downregulate mRNA targets. Argonaute proteins (e.g. Ago2) play a major role in the knock down of gene expression by the RISC complex, mediating selective endonucleolytic cleavage of target RNAs. The successful avoidance or downregulation of the RNAi machinery is vital for arboviruses to productively infect their arthropod vectors. There are several reports to identify individual susceptibility markers, recombinant antibodies, peptides, RNA interference, and small molecules with the ability to directly or indirectly neutralize WNV. Till now effective therapy is still lacking [83].

A recent approach to search for new antiviral agent candidates is the assessment of long-used drugs commonly administered by clinicians to treat human disorders, as part of drug repositioning (finding of new applications to licensed drugs). Among some of the drugs already tested as antivirals are lithium, statins, or valproic acid [84,85]. Since there is evidence supporting that WNV infection shares common points with Parkinson's disease, study was undertaken whether drugs used for the treatment of Parkinson's disease could provide novel tools for antiviral intervention. In this way, assessed the effect of four antiparkinsonian drugs (L-dopa, Selegiline, Isatin, and Amantadine) in WNV multiplication in cultured cells from different origin. L-dopa, Isatin, and Amantadine treatments significantly reduced the production of infectious virus in all cell types tested, but only Amantadine reduced viral RNA levels. This results indicates that Amantadine, as possible therapeutic candidates for the development of antiviral strategies against WNV infection.

There are recently four USDA-licensed vaccines available for equines in which two are attenuated whole WNV one is a non-replicating live canary pox recombinant vector vaccine, and another is an attenuated flavivirus chimeric vaccine. Passive immunization has been used in a some cases; Sometimes it also causes allergic reactions. A case study of two WNV encephalitis patients treated with alpha interferon, the standard of care for infection with the related flavivirus hepatitis C virus, showed significant melioration and an improved recovery course. Several strategies are being used for the advancement of a vaccine in humans that may significant for use. Reports include live inactivated vaccines, recombinant subunit vaccines, vectorized vaccines, DNA vaccines with constructs that express the WNVE protein, live recombinant vaccines, and an inactivated strain based on nonglycosylated E and mutant

NS1 protein [86]. A neutralizing WNV-specific monoclonal antibody E16 (MGAWN1), which enters in the CNS in animal models, generates neutralizing antibodies in phase I trials. Very effective results were seen with a chimeric vaccine based on the WNV prM and E proteins inserted into the yellow fever 17D vaccine moiety (ChimeriVax-WN02). It was shown to be effective, safe and immunogenic in phase II clinical trials, with high seroconversion rates, yet it is no longer available [87,88].

12. Preventive measures and control

Prevention and control of WND require strategy that includes vaccination, mosquito control and clinical management. Preventive tools are necessary for inhibiting WNV infections other than drugs and vaccines can also aid to armed the transport of the infection by avoiding mosquito bites [89]. These simple measures can be summarized under the title 'fight the bite!' and consists of the use of insect repellents, the removal of standing water where mosquitoes lay eggs, the reduces the outdoor activities cooccurence with the maximum activity of mosquitoes, reporting dead birds to local authorities and helping mosquito control programs [90].

13. Future Directions

WNV has now persisted and causes endemics in North America. Transmission of the mosquito vectors harboring WNV to include *Aedes albopictus*, a mammal-biting mosquito. It is believed that the increase in our knowledge of WNV with the mosquito vector will lead to new boulevard for therapeutics and preventive measures. Mosquito responses at the levels of protein and gene expression [91]. Many strategies to novel targets to concentrate our efforts to prevent or block WNV infection. For example, a single-chain human monoclonal antibody developed through phage display against the envelope protein reveled both protection and therapeutic efficacy when detect in the murine model. Current advancement in nanoparticle technology have also been used in vaccination studies of murine WNV infection and show significant efficacy of TLR9-targeted biodegradable nanoparticles, which produce a large number of circulating effector T cells and antigen-specific lymphocytes . Potency to relevant viral susceptibility mechanisms, processing host antagonism of chemokine responses as has been noted in infection with the related flavivirus hepatitis C virus.may show infectious mechanisms used by WNV. The rate of vector discovery, virus, and host molecules of pathogenesis provide critical insights for the controls and therapeutics for WNV [92].

14. Conclusion

The recent amanation and transport of WNV in America and increase in number and severity of outbreaks in Europe shown one of the major zoonotic treats in years. Although our information about WNV infection has increased in recent years, some panorama of WNV activity still essential to be further addressed: the ways by which WNV colonizes new habitats

and the role that climatic (temperature, humidity, *etc.*) factors play; the differences in WNV disease demonstration between the US and other parts of the world, mainly Central and South America; a better knowledge about WNV immunity, pathogenicity, and the factors which causes virulence; the long-term manifestations of WNV infection and the results of persistent infections; the progression of national and international surveillance programs to monitor WNV outbreaks and to take appropriate strategy to control it. The search for more efficient, rapid, and specific and sensitive diagnostic assays that can be easily adopted in all over the world; and the search for cheap human vaccines for high risk targeted populations and for new antiviral targets for therapeutic usage. Advancement on our current knowledge on WNV infection will greatly help to fight not only future transmission of WNV to habitats around the world, but also of other Flaviviruses.

15. Acknowledgment

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