Immunopathogenesis of West Nile Virus Infection

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Abstract. The reappearance of West Nile virus (WNV) infection in the last years has highlighted that arthropod-borne diseases are not circumscribed to tropical regions of the world. WNV is maintained in enzootic cycles involving Culex spp. mosquitoes and avian hosts, with epizootic spread to mammals, including humans. Human infection results in mild symptomatic illness in 25% of cases or neurological disease in less than 1% of infected persons. Additional understandings on how WNV interacts with its hosts are recently emerging; the virus exploits immune system, both at the peripheral tissues and the central nervous system, which could explain the differences in virulence, progression and severity of WNV infection. The continuing spread of WNV, combined with the lack of specific therapeutics or vaccines to combat or prevent infection, imparts a pressing need to identify the viral and host processes that control the outcome and immunity to WNV infection. Here, we provide an overview of a subset of information regarding the immune-pathological response generated during WNV infection.

Keywords: cytokines, viral infection, inflammation, T cells.

Imunopatogénesis da infeção pelo vírus do Nilo Occidental

Resumo. O ressurgimento da infeção pelo Virus do Nilo Ocidental (VNO) nos últimos anos tem demonstrado que as doenças transmitidas por artrópodes não estão vinculadas às regiões tropicais do mundo. O VNO mantém-se em ciclos enzoóticos que envolvem mosquitos do gênero Culex spp. e hospedeiros aviares, com distribuição epizootica em mamíferos, incluídos os seres humanos. A infeção em seres humanos ocasiona doença sintomática leve em 25% dos casos e doença neurológica em menos de 1% das pessoas infectadas. Recentemente, tem surgido outros entendimentos sobre a forma na qual o vírus interage com os hospedeiros; o vírus aproveita-se do sistema imune, tanto no sistema nervoso central, o que poderia explicar as diferenças na virulência, evolução e gravidade da infeção pelo VNO. A continua propagação do VNO, junto com a falta de terapias ou vacinas específicas para combater ou prevenir a infeção, gera a necessidade de identificar os processos virais e do hospedeiro que determinam o pronóstico e a imunidade à infeção pelo VNO. No presente artigo, oferecemos uma visão geral de informação a respeito da resposta imunopatológica gerada durante a infeção pelo VNO.

Palavras-chave: citocinas, infecção viral, inflamação, linfócitos T.
Introduction

West Nile virus (WNV) is a member of the Flaviviridae family, which also includes dengue virus, yellow fever virus, Japanese encephalitis virus and St. Louis encephalitis virus, among others, that causes illness in humans [1]. WNV was first isolated in the West Nile region of Uganda in 1937 [2] and, since the mid-1990s, outbreaks of WNV infections have occurred in regions all around the world, including the Middle East, Europe, and Africa [3]. After its introduction into USA in 1999, WNV rapidly disseminated across North America, South America, and the Caribbean [4, 5].

In most cases, WNV infection in humans is asymptomatic or has mild unspecific symptoms. Almost 25% of infected people develop West Nile fever with malaise, headache, myalgias and lymphadenopathy. In about 1% of the WNV infection cases, severe neurological complications, such as encephalitis, meningitis or acute flaccid paralysis are seen, carrying a fatality rate of approximately 10% [6, 7]. In the latter group, mortality rate can reach 20% and, in those who survive, neurological and functional disability represents a considerable source of morbidity long after the onset of acute illness [8, 9]. The main risk factor for neuroinvasive disease is advanced age, while diabetes mellitus, excessive alcohol, and cancer may also increase risk [10]. At least a 20-fold increased risk has been reported in patients older than 50 years of age [11, 12]. In addition, a limited number of host genetic factors have been linked with susceptibility to WNV infection. For instance, a deficiency of the chemokine receptor CCR5 increases the risk of early symptomatic WNV infection [13].

In addition, single nucleotide polymorphisms (SNPs) in IRF3 and MX1 have been associated with symptomatic WNV infection and a single SNP in 2’-5’-oligoadenylate synthetase/1.1 (OAS1) was associated with increased risk for West Nile encephalitis and paralysis [14]. Despite the high economic and social impact and growing public health concern, current treatments for WNV are inadequate. While effective WNV vaccines are widely available for horses, no human vaccine has been registered [10].

WNV replicative cycle

WNV is an enveloped virus with a single-stranded RNA genome with positive polarity. Several molecules have been implicated in the attachment of WNV to cells in vitro, including DC-SIGN, DC-SIGN-R, the integrin αβ₃ [15], NKp44 [16] and mosgctl-1 (a C-type lectin in mosquito cells) [17]. The virus enters target cells by endocytosis and, after membranes fusion, the viral RNA is released into the cytoplasm [18]. In the cytoplasm, the RNA genome is translated into a polyprotein that is cleaved by proteases from virus, to obtain three structural proteins, c (capsid), prM (membrane) and e (envelope), and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [18]. The RNA-dependent RNA polymerase, NS5, synthesizes the complementary minus-strand RNA molecule from the genomic RNA, which subsequently acts as a template for the synthesis of new positive-sense viral RNA [19]. WNV assembles in the endoplasmic reticulum to form immature particles containing the prM protein. Following transport across the trans-Golgi, mature infectious virions are generated and released by exocytosis [18, 20, 21].

Ecology of WNV

WNV is maintained in an enzootic cycle between mosquitoes and birds; however, humans, horses and other mammals can also be infected [22]. The virus possesses different vectors depending on the geographical region, but the most important vectors are mosquitoes of the Culex genus [22]. Several avian species are highly susceptible to WNV and are the most important reservoirs in nature; during a time window of 3–7 days after infection, the WNV titers are high enough to transmit the virus to blood-sucking mosquitoes and infect them. Nevertheless, in rare cases, WNV transmission has been documented among humans via blood transfusion and organ transplantation [23] and even from a mother to her newborn via the intrauterine route [24] or via breast-feeding [25].

Immunopathogenesis

After mosquito inoculation, WNV replicates in keratinocytes, neutrophils and Langerhans cells. Infected dendritic cells migrate to draining lymph nodes. Here, infection and the risk of dissemination are frustrated by the rapid development of an early immune response including type I IFN production and the effector functions of innate immune cells (γδ T cells, macrophages, NK cells, neutrophils and IγM-secreting B cells) [26-28].
In mouse models, γδ T cells have been shown to expand as early as day two post wNV infection and to be indispensable for controlling its replication and dissemination through the production of IFN-γ [29, 30] (figure 1). In addition, γδ T cells may contribute to the cytotoxic activity against wNV in the periphery directly and/or indirectly by regulation of αβ T cell response [31]. Besides, γδ T cells promote the maturation of dendritic cells during wNV infection, favoring adaptive immune responses [32].

Figure 1. wNV pathogenesis. a) Upon mosquito bite, wNV is inoculated in the intradermal space where infects keratinocytes, resident macrophages (mf) and dendritic cells (DC). At this level infection and dissemination is controlled by type I interferons (IFN-a/β) produced by cells from the innate immunity. wNV induces expansion of γδ T cells which in turns favors DC maturation, IFN-γ production and finally control virus replication. As DCs get infected, wNV disseminates to lymph nodes where more replication occurs allowing the spread to peripheral organs such as spleen and liver. b) Infection of peripheral organs is controlled by serum I y M (in red), the cytotoxic activity of CD8+ T cells and IFN-a/β produced by resident macrophages. Whereas spleen is a permissive organ for wNV replication, liver is not. This phenomenon has been associated with the presence and activity of NK cells in the liver. The infection is further amplified and the virus might reach the central nervous system (CNS). c) wNV employs several mechanisms to cross the blood-brain barrier (BBB), among them, crossing through endothelial tight junctions (left); infecting leukocytes that traffic to the CNS (middle) and by direct infection of the brain microvascular endothelial cells. Source: Compiled by the authors

Innate immune response, including antiviral factors, type I IFN and innate cell-mediated responses, orchestrates the early control of wNV, whereas adaptive immune response, including humoral and cellular responses, is essential for wNV clearance and inflammation-associated damage at the end of infection [33].

After wNV replication, virions enter circulation causing primary viremia, which allows spread to secondary lymphoid and visceral organs including spleen and kidney [28]. After that, the wNV infection can be restricted by immune responses including antibodies, type I and II IFN and cytotoxic CD8+ T cells, among
others [34-36] (figure 1). Interestingly, while the liver is exposed to WNV, the infection seems to be controlled by innate immune response mediated by RLR and type I IFN-dependent signaling. Suthar et al [37] have applied a mouse whole-genome microarrays to profile global gene expression changes between permissive (spleen) and non-permissive (liver) tissues from wild type infected mice. The NK cell signaling canonical pathway was exclusively enriched in the liver, suggesting that NK cells signaling pathways play an important role in the control of WNV infection in liver (figure 1). Also, using a model of knockout mice for the IFN-α and MAVS (and adaptor protein downstream the RLR signaling) the authors show the importance of these innate factors for regulating NK cell functions in the liver of WNV infected mice [37].

If the infection cannot be controlled, the virus will be amplified in spleen and, following a secondary viremia, WNV may cross the blood-brain barrier (BBB) and enter to central nervous system (CNS; figure 1). WNV can enter the brain through many routes including a) endothelial tight junctions, whose permeability might be compromised by pro-inflammatory cytokines like TNF-α or the action of matrix metalloproteinases; b) direct infection of brain endothelial cells; c) infected leukocytes that travel to the CNS; d) infection of olfactory neurons; and e) retrograde axonal transport from infected peripheral neurons, as reported in mouse models [38, 39]. Regardless of the route of entry, once inside the CNS, the main cell target of WNV is neurons from multiple regions of the brain, although other cells, such as myeloid cells and possibly astrocytes, can also be infected [39]. The development of encephalitis is mediated through direct damage of cells induced by the virus itself and through indirect mechanisms mainly involving inflammatory response.

The direct mechanism responsible for neural injury is the apoptosis induced by caspase 3 or other proteases (e.g., calpains and cathepsins) in the cerebral cortex, brain stem, and cerebellum [40]. Although the precise mechanisms by which WNV induces apoptosis are not completely understood, it has been shown that viral proteins like NS3 and Capsid C induce apoptosis by activating caspases 8 and 3 [41, 42]. Also, Balmori et al [43] have suggested a possible role of the protein NS2A in the IFN-independent apoptotic cell death. The authors have shown how a single amino acid (alanine or lysine) might control the cytopathic effect and apoptosis in the absence of IFN-α/β response. Besides apoptosis, WNV also induces necrosis, especially in infections in which there is an extensive viral production and the WNV virions accumulate in the endoplasmic reticulum [26].

In addition to direct cell damage, the release of inflammatory molecules (e.g., CXCL1, IL-1β, IL-6, IL-8, and TNF-α) from dying neurons can have toxic effects on uninfected neurons. Furthermore, glial cells, which are not primary targets of WNV infection, can be activated and release reactive oxygen species and pro-inflammatory cytokines that contribute to WNV pathogenesis [44]. Moreover, other components of immune response, like IL-22, have been shown to be harmful for the host during WNV infection. Wang et al [45] described how this cytokine facilitates the entry of WNV into the CNS through the induction of CXCR2 expression on leukocytes and, of CXCL1 and CXCL5 on endothelial and brain cells. CXCR2 is a chemokine receptor that mediates neutrophil migration, and CXCL1 and CXCL5 are the ligands. The authors described how the presence of IL-22 facilitates neutrophil infiltration into the CNS, and those neutrophils could carry WNV to cross the BBB and could contribute to CNS inflammation and BBB disruption by producing inflammatory mediators such as matrix metalloproteinases [45].

In the same line of evidence, the subset of γδ T cells named Vγ4+ mediate pathogenic effects through the production of TNF-α. It has been described that Vγ4+ cell-depleted mice had reduced TNF-α levels in the CNS, accompanied by a decreased viral load in the brain and a lower mortality to WNV encephalitis [46].

There must be a refined immune response aimed at limiting the spread and eliminating WNV without neural damage. Type I IFNs have been shown to be important for WNV clearance from CNS and control of viral spread. However, WNV counteracts this arm of the immune response. NS2A inhibits IFN-β transcription and expression, while NS4B and NS5 block IFN signaling pathways by inhibiting STAT1 and STAT2 or JAK1 and TYK2 phosphorylation, respectively, downstream of type I IFN expression [47]. Therefore, big players in the clearance of the virus are CD8+ T cells. However, these cells can also cause irreversible damage to the host and contribute to WNV pathogenesis [48]. Regulatory T cells constitute a way to control the excessive activity of CD8+ T cells and, interestingly, it has been reported that high Treg levels are associated with asymptomatic WNV infections [49].

Finally, WNV has evolved mechanisms to counteract immune response. WNV antagonizes or evades detection by RLRs [50-52], which allows the virus to establish infection and synthesize viral factors to hamper
other downstream innate immune signaling pathways. WNV has also been shown to interfere with type I IFN response [53-55]. Several WNV proteins have been implicated in the antagonism of the type I IFN signaling cascade. Both structural and non-structural proteins of WNV suppress type I IFN signaling [56, 57].

Conclusion

Because most of the WNV infections are asymptomatic, we can be sure that immune response to the infection is efficient enough. Many factors can account for the development of neuroinvasive disease, including inoculation dose, WNV strain, age, and immune status, among others. Only a better understanding of the immune mechanism underlying asymptomatic and symptomatic infections will help us in the development of specific therapies or vaccines, or at least will help us to predict whether immunopathology will occur and therefore be better prepared for the eventuality of an epidemic.

Disclosure of Conflicts of Interest

None of the authors has any potential financial conflict of interest related to this manuscript.

References


