MiniReview

New insights on the neuropathogenicity of West Nile virus

Pierre-Emmanuel Ceccaldi a,*, Marianne Lucas b, Philippe Despres b

a Rabies Unit, Virology Department, Pasteur Institute, Paris, France
b Host-Flavivirus Molecular Interactions Unit, Virology Department, Pasteur Institute, Paris, France

Received 9 December 2003; received in revised form 20 January 2004; accepted 20 January 2004

First published online 6 February 2004

Abstract

West Nile virus (WNV) is a mosquito-borne disease that emerged in North America where it caused in 2002 the largest arboviral meningoencephalitis outbreak ever recorded in this area. The viral variant responsible of this outbreak has been found to share 99.7% identity over the entire genome with the viral variant that caused the epizootic in Israel in 1998 and has been referred as “Isr98/NY99”. It has been shown to exhibit an increased neurovirulence in humans, as well as in experimental infections in different animal models. Mouse model has allowed to demonstrate the preferential infection of neurons within the central nervous system and to point out the genetic determinism of host susceptibility to WNV. In murine neural cell cultures, the selective infection of neurons was accompanied by physiopathological changes and a cytopathic effect, showing the direct effect of infection of neurons as one of the causes of WNV neuropathogenicity.

© 2004 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Zoonosis; Arthropod-borne viral disease; Flavivirosis; Emerging disease; Viral encephalitis; West Nile virus; Genetic susceptibility

1. Introduction

West Nile virus (WNV) is a single-stranded RNA virus of the Flaviviridae family, genus flavivirus. It was first isolated from a person with a non-specific febrile illness in Uganda in 1937 and has been recognized as endemic since the 1950s in the Middle East. Since 1996, several outbreaks occurred in Romania, Russia, Israel, and, more surprisingly, in United States of America where it appeared for the first time in 1999 (Table 1) [1,2]. West Nile outbreaks in recent years have coincided with the emergence of a new variant of WNV designated “Isr98/NY99” that circulates in North America and the Middle East [3]. Notably, Isr98/NY99 was characterized by a high avian death rate and an apparent increase in human disease severity with 13,000 cases and 500 deaths in USA since 2002 (Table 2) [4], consistent with the hypothesis that some changes in the neurovirulent properties of the virus had occurred [5,6]. The increased neurovirulence of Isr98/NY99 is accompanied by several novel modalities of transmission to humans, besides the classical mode of mosquito bite: transplacental transmission to the fetus, transmission via breast milk, blood transfusion, or laboratory contamination through percutaneous inoculation [1,6].

Emerging infectious diseases can be a completely new disease or an old disease occurring in new places or new species through host-switching. The recent WNV outbreak in North America concerns the introduction into new areas of a previously known aetiological agent, but with increased neurovirulence [7]. The present review focuses on the neuropathogenic properties of Isr98/NY99 that has been responsible for the recent WN outbreaks.
NY99 could be characterized by an ability to infect a spinal cord, although at a low frequency [11]. Viral antigens could be detected within the brain and the exhibited neurological signs (paraplegia) confirmed that study in naturally WNV-infected horses in Israel that neuronal cytoplasm and fibers. At the same time, a be detected in glial cells, macrophages and within the brainstem and spinal cord, scant WNV antigens could histopathological lesions within the basal ganglia, glial nodules, and some neuronophagia [10]. Besides these characteristics have been reported in humans for the WNV Isr98/NY99 in the past few years [1,4].

The neuroinvasiveness of WNV Isr98/NY99 in humans has been demonstrated by the first isolation of infectious WNV from a patient with encephalitis in the United States [13], and by immuno-histochemistry and PCR investigations on brains from four first lethal human cases of encephalitis in New York in 1999, that showed WNV brainstem and spinal cord infection [14]. Neurotropism could be demonstrated indirectly by observation of motor neuron losses in the spinal cord of infected patients and signs of axonal neuropathy [14–16], and by detection of WNV antigens within the cytoplasm of brain neurons from a patient that died of WNV fulminant pan-meningo-polioencephalitis [17]. Among numerous clinical studies of WNV infections in humans, in United States as in Israel, the neurovirulent properties of Isr98/NY99 were clearly demonstrated, as reported by Weiss et al. [18], that underlined the predominance of gastrointestinal and neurological symptoms in hospitalized patients in New York and New Jersey in 2000. Interestingly, these authors found that in humans, disease severity was associated with age, with a mean of patients of 71 years, whereas another study pointed out an age of 75 years old or older as an independent risk factor for death [16]. Among the neurological manifestations that are observed in human cases, rare seizures can occur but a high incidence of cranial nerve dysfunction has been reported, suggesting involvement of brainstem during infection [15]. More recently, converging reports highlighted the link between WNV Isr98/NY99 infection and flaccid paralysis and muscle weakness; and it is important to note that these symptoms may occur in the absence of fever or meningoencephalitis signs [6,19].

### 3. Neuropathogenicity in humans

CNS infection by a neurotropic virus can be characterized by three different properties: neuroinvasiveness (the ability to enter the CNS), neurotropism (the ability to infect neural cells), and neurovirulence (the ability to cause neurological syndrome). As underlined below, all these characteristics have been reported in humans for the WNV Isr98/NY99.
WNV-induced polio-like syndrome is mainly characterized by an asymmetrical muscle weakness, reflex loss and motor axonopathy without sensory nerve involvement, that would suggest anterior horn cell loss [20]. Also, histopathological studies showed in WNV-infected patients with quadraplegia and loss of brainstem function the presence of microglial infiltrates especially in the vicinity of anterior horns [21], with signs of motor neurons destruction in this region [22,23]. It should be noted that among 50 patients of the 1999 New York WNV outbreak, 12 of them exhibited muscle weakness, and six had flaccid paralysis; electrophysiological studies in these patients confirmed involvement of anterior horns, with loss of motor units and evidence of collateral reinnervation (giant motor unit potentials) [16,24]. In conclusion, the study of human cases of WNV infection allowed to confirm the high neurovirulence of WNV Isr98/NY99, the existence of age-related factors in neuropathogenesis, and the selective vulnerability of some CNS regions, such as the spinal cord, with possible recovery; however, the question of viral persistence within the CNS is not yet cleared, although WNV-reactive serum IgM antibodies could be detected in confirmed human cases of WNV encephalitis as late as 1.5 years after onset [25].

4. Neuropathogenicity in animal models

As one of the main natural targets of WNV infection, birds have been chosen as animal models for WNV infection. In an extensive study, Komar et al. [26] exposed 25 bird species to WNV-infected mosquito bites. In infected birds (28/87), neurological signs could be observed, such as inability to hold head upright, lethargy, ataxia, and unusual posture. Vocal load in the different organs pointed out prioritization of brain and kidney for WNV infection. This study also demonstrates the existence of viral persistence in some birds that survived and differences in susceptibility among the species [26].

Although avian models of WNV infection are merely representative of natural infections, other animal models, more convenient for neuropathogenic studies, have been developed in several laboratories. In fact, most of studies in the past that concerned the primary strains of WNV that had been isolated previously to WNV Isr98/NY99 had been performed on the mouse model, leading to delineate the neurotropic and neuroinvasive properties of these viral isolates (for example, see [27]). It could be demonstrated in a hamster model of WNV Isr98/NY99 infection that after intra-peritoneal inoculation, a low duration viremia (5–6 days) was followed by the first signs of encephalitis [28]. In this rodent model, neuronal degeneration with viral replication in the basal ganglia occurred as early as day 5 post-inoculation. Two days later, WNV infection was detected in neurons of the cerebellar cortex, frontal and parietal cortices, and hippocampus. The fact that large neurons from the anterior horn of the spinal cord were positive for viral antigens late in infection is in agreement with some relevance of the model with human infection, especially for what concerns the polio-like syndrome as quoted above. Interestingly, WNV was recovered up to 2 months post-inoculation, suggesting that persistent infection can take place in convalescent animals [28]. In another study, hamster and mouse models have allowed to determine that particular WNV strains from North America were highly neuroinvasive [29] and infection was associated with a loss of age-related resistance that had been observed for previous isolates of WNV. A mouse model of WNV infection has shown the neuroinvasiveness and neuronal tropism of WNV Isr98/NY99 whatever the route of infection [30–33] and unpublished results (Fig. 1). The mouse model was also used to point out the role of humoral immune response in limiting the spread of WNV infection in the CNS after primary replication in the lymph nodes [34], the role of CD8+ T cells in both recovery and immunopathology [35]. Recently, this model of infection has allowed to demonstrate that passive transfer of immune antibodies could improve the clinical outcome even after WNV had reached the CNS, although antibodies by themselves could not completely eliminate viral reservoirs in host tissues [36]; in this context, Diamond et al. [37] have recently demonstrated the role of specific anti-WNV neutralizing IgM in preventing CNS infection and viral-induced death. The mouse model was also used to study the genetic determinism to the susceptibility to WNV
infection. We and others reported that a nonsense mutation in the interferon (IFN)-inducible gene encoding the 1b isoform of the 2′,5′-oligoadenylate synthetase (Oas) was associated with the susceptibility of mouse strains to experimental infection with WNV [30,38].

In conclusion to these different experimental infections that were performed, it has been shown that infection of birds, as natural reservoirs of WNV, provide evidence of variations in neuropathogenicity among different species. It is still unknown whether host-dependent genetics might result in differential susceptibility to WNV infection in birds. In the meantime, murine models, that have allowed to conduct these genetic studies, also pointed out the neuroinvasiveness of WNV, the preferential infection of neurons within the CNS and the possible persistence. The WNV strains that were recently isolated in North America and Israel were found to be highly neuroinvasive in mice with a lack of age-related resistance, an unusual phenotype among encephalitic flaviviruses. The reasons for this change are unknown and may be the result of differing properties of Isr98/NY99 compared to WNV strains primarily isolated in the Old World [39].

5. Neural cell cultures

The marked neurotropic properties of WNV in natural infections in humans or in animals, as in experimental infections, led to the development of neural cell cultures in order to better delineate the pathogenic mechanisms of WNV infection. We performed neural cell cultures from brain cortex of embryonic brain cortex (day 15 of gestation) [40]. Neural cell cultures were done according to two different protocols that allow either enrichment (more than 90%) in astrocytes or in neurons. Even at high multiplicities of infection (100), very low level of infection was detected in astrocyte-enriched cultures (less than 10% of cells infected). On the contrary, neuron-enriched cultures were shown to be infected for WNV infection (Fig. 2), and at 48 h post-infection more than 90% of the neuron-enriched cultures could be infected (unpublished data). WNV infection of neurons was accompanied by cytopathic changes and cell mortality (unpublished data). The role of genetic factors in WNV neuropathogenicity could be confirmed in these neuronal cultures, since WNV replication was less efficient in cells that produce the normal copy of Oas1b as compared to those expressing the inactive mutated form [40]. It is noticeable that although other cell types should be considered to play a role in the neuropathogenicity, such as immune cells [34,35], the role of host genetic factors could also be demonstrated in neuronal cell lines (Neuro2a), where WNV infection was less efficient in normal cell clones that overexpress wild-type Oas1b as compared to those that express the inactive form [40]. In order to determine whether neuronal injury was provoked by viral infection or by the immune response, a culture model from embryonic stem cells has been recently developed by Shrestha et al. [32] who demonstrated that although embryonic stem cells were relatively resistant to WNV infection before differentiation, they became permissive for WNV infection once differentiated, and further die by apoptosis. This could be linked to our recent data suggesting an important role for the M ectodomain in WNV-induced apoptosis [41]. WNV-induced cell death by apoptosis in cultured cells had already been demonstrated in non-neuronal cells (Vero cells) for low multiplicities of infection, although higher multiplicities of infection could induce morphological changes characteristic of necrosis as early as 8 h post-infection [42].

Primary cultures of mouse embryonic neurons have also allowed us to study some dysregulations of gene expression that could occur during WNV infection. The cDNA expression array hybridization method was used to identify genes that were differentially expressed in primary neuronal culture infected by WNV. Late in infection, we observed that genes encoding c-jun and jun-D transcription factors were up-regulated in response to WNV replication (unpublished data). The fact that dysregulation might occur on mouse neuronal genes such as transcription factors indicate that WNV is able to induce physiopathological cascades directly by infecting neurons, even before the first cytopathic effects could be observed. In this context, nine genes have been reported to be up-regulated in the central nervous system of Japanese Encephalitis virus-infected mice; among them, transcription factors genes such as STAT1 were found to be concerned, as well as Oas gene, that were also found to be involved during rabies virus infection in mouse [43]. Thus, the physiopathological cascades that
occur during WNV infection could be linked to a more general pattern of neurotropic viral infections, and primary cultures of neurons provide a convenient support for studies of genetic susceptibility or for comparative studies of different WNV variants.

6. Conclusions and perspectives

In spite of numerous studies that were published in the past few years, that have brought evidence that Isr98/NY99 WNV variant has higher neurovirulence and neurocytopathogenicity compared to the previous isolates of WNV that had been studied, some major questions still need to be elucidated:

- what may explain the increased susceptibility that is linked to the age: in particular the influence of age on blood–brain barrier characteristics or antibody production should be addressed.
- the role of host genetic factors: demonstration in a mouse model of a genetic determinism in the susceptibility to WNV will lead to search for a correspondence in human cases; especially, it would be interesting to study whether the human genetic polymorphism might be critical in WNV pathogenesis.
- WNV infection of the neurons of the CNS raises the question of a possible persistence of WNV; in particular, the report that WNV-reactive serum IgM antibodies could be detected in confirmed human cases of WNV encephalitis as late as 1.5 years after onset [24] could be of great concern in public health.

Clearly, a more complete understanding of the mechanisms responsible for WNV encephalitis will require in the next few years studies on the innate susceptibility and the protective effect of the adaptive immune responses in WNV infection [44].

Acknowledgements

M.L. is post-doctoral fellow of the Transverse Research Program (Institut Pasteur; Paris). Marie-Pascale Frenkiel is acknowledged for skillful technical assistance.

References


