EDITORIAL

The Emergence of West Nile Virus in Canada

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In 1999, several cases of encephalitis due to West Nile virus (WNV) were recognized in New York City.[1] The appearance of WNV in North America represents a dramatic departure in the geography and ecology of a neurovirulent arbovirus, moving from the Eastern to the Western Hemisphere with an ensuing continent-wide epidemic.[2] West Nile virus infection results in significant neurological disability and death in a subset of infected patients.[3] All levels of the neural axis are infected by WNV, manifested by a myriad of signs and symptoms. The papers by Burton et al[4] and Sayao et al[5] in this issue of the Canadian Journal of Neurological Sciences extends our understanding of the neurological aspects of WNV infection by describing neurological cohorts from Toronto and Calgary respectively, including several patients with marked systemic immunosuppression. Although the WNV epidemic is a recent phenomenon in North America, its neurovirulent properties in humans and other species including birds and horses have been evident for some time. West Nile virus was originally isolated from the blood of a 37-year-old febrile woman in the West Nile province of Uganda and was recovered by the intracerebral inoculation of mice.[6] Paradoxically, WNV encephalitis in humans was initially described in New York City when the virus was inoculated into 95 patients with advanced cancer, assuming that it might have oncolytic effects. Subsequent encephalitis developed in nine patients, from which virus was recovered in the spinal fluid of three patients. The first major European epidemic occurred near Bucharest, Romania, in 1996 in which 835 patients were admitted to hospitals with apparent nervous system infections among which 80% were shown to have WNV infection with a case-fatality rate of 10%. In the same year, an outbreak in Volgograd, Russia resulted in 826 admissions to hospitals for nervous system syndromes including 84 patients with severe meningo-encephalitis, of whom 40 died.[3]

The virus is an RNA virus belonging to the flavivirus family[7] and is a member of an antigenic complex of Flaviviridae that includes St Louis encephalitis, Kunjin, Murray Valley encephalitis, and Japanese encephalitis virus. West Nile virus has been recovered from a variety of urban mosquitoes, but members of the *Culex* genus appear to be the prime vectors, due to their potential role in the observed overwintering of WNV in temperate climates.[2] Horses, like humans, are considered dead-end hosts, and numerous cases of equine WNVencepahlitis have been reported, while other mammals are also thought to be incidental hosts. In addition, WNV has been shown to be transmissible by blood transfusion and organ transplants.[8] Most WNV infections are asymptomatic or subclinical (80%), while the remaining 20% exhibit fever, headache, anorexia and general malaise that develop abruptly without prodromal symptoms.[9,10] Neurological complications have been reported with variable frequency, usually in approximately 1% of all cases.[11] Aside from meningo-encephalitis that appears to involve chiefly the subcortical structures including the basal ganglia, thalamus and brainstem, flaccid paralysis resembling poliomyelitis or Guillain-Barré syndrome have been described in a number of patients[12] and are extended by the current papers by Burton et al[4] and Sayao et al.[5] The paper by Burton et al[4] also provides important electrophysiological analyses of neuromuscular involvement. Cranial MRI studies, albeit few published, show lesions, frequently symmetric in the above subcortical regions.[13] Of note, the papers by Burton et al[4] and by Sayao et al[5] include high quality neuroimaging of WNV neurological disease that is not widely available in the literature.

While the numbers of infected patients has steadily increased in North America over the past three years, there has been limited progress in understanding underlying neuropathogenesis of WNV. The virus infects both neurons and glia within the central nervous system with evidence of WNV antigens (capsid and envelope protein) primarily in neurons, although glial cells are also WNV immunopositive.[14,15] Infection of the CNS results in marked neuro-inflammation including mononuclear cell infiltrates, microglial nodules, perivascular cuffing, edema together with neuronal death and neuronophagia primarily in the gray matter. The roles of individual viral proteins in the neuropathogenesis of WNV remain uncertain but the data collected so far suggest there is limited molecular heterogeneity among different WNV strains, unlike other RNA viruses including another flavivirus, hepatitis C virus and human immunodeficiency virus. Sequence analyses of the WNV isolates from birds and humans in the USAindicate that all share a common origin, and are closely related to the first North American isolate, termed the NY99 strain.[16] Indeed, brain-derived WNV capsid sequences from two fatal cases described by Burton et al[4] and Sayao et al[5] suggest that the virus exhibits minimal evolutionary divergence from the original NY99 strain (van Marle and Power, unpublished findings). The North American WNVis most closely related to a virus isolated from a dead goose in Israel in 1998 (99.8 percent identity).[1] The limited molecular diversity within WNV bodes well for vaccine development, which is currently in use for horses, birds and other lower species although human vaccination is still several years away.

Based on studies of other neurovirulent flaviviruses including Dengue and Japanese encephalitis viruses, the likely candidate WNV proteins responsible for driving neurovirulence include the envelope (E) and capsid (C) proteins.[17] Robust host humoral responses to WNV have been documented in different species[18] with the envelope protein appearing to be the principal target for neutralizing antibodies.[19,20] To date, a defined cellular receptor for WNV remains unknown, although recently a 105 kD cell surface glycoprotein has been proposed.[21] Moreover, infection is modulated by interferon-inducible 2'-5' oligoadenylate synthetase-1b protein.[22] The latter observation, also underscores

the potential therapeutic use of IFN-α, as suggested in the current paper by Sayao et al.[5] West Nile virus infection is mediated through binding of the glycosylated E protein to the cell surface receptor and subsequent endocytosis of the virus particle through pH-dependent conformational changes in the E protein, leading to fusion with cell membranes.[23] The mechanism of neuronal death is assumed to be through apoptosis although the precise death signaling pathways remain uncertain but a recent report suggests high viral load may also push the mechanism of cell death to a necrotic phenotype in non-neuronal cells.[24] Earlier studies suggested that WNV-mediated apoptosis in K562 and Neuro2a cells, via activation of Bax.[25] Expression of caspase 9 was induced by the WNV capsid protein in HeLa cells and *in vivo* after implanting a WNV capsid protein-encoding expression vector into the brain.[26] At the same time, it is apparent that innate immunity participates in terms of glial cell activation with consequent release of potential neurotoxins. West Nile virus has been shown to regulate MHC Class I expression in mouse fibroblasts via NF-κB activation[27] and WNV infection of astrocytes down-modulates MHC class I expression.[28] Similarly, the capsid protein was able to induce a Th1 response in mice.[29] To date the role of selective adaptive cellular immunity has not been consistently demonstrated in WNV infections.[30,31] Of interest, macrophage activation by LPS results in increased WNV neurovirulence in mice, which was not blocked by anti-TNF-α antibodies.[32] Macrophage depletion leads to enhanced WNV neuroinvasion and neurovirulence, highlighting the importance of these cells in controlling infection.[33] Finally, it is noteworthy to mention the ability of WNV to infect Schwann cells, which may be an important pathogenic prerequisite with regard to the demyelinating neuropathies observed by Sayao et al.[5]

With the emergence of WNV in Canada, health care providers will need to be increasingly vigilant in identifying patients with WNV-related neurological diseases, chiefly in the summer months. Patients presenting with fever, cerebrospinal fluid pleocytosis and distinctive neurological syndromes, especially those with concurrent immunosuppression will require intensive evaluation to exclude WNV infection. Therapeutic options for WNV-related neurological diseases are few and are discussed in an accompanying editorial.[34] However, given the magnitude of the epidemic and minimal understanding of WNV neuropathogenesis, Canadian clinicians involved in neurological care are likely to encounter these syndromes with increasing frequency over the next few years.

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