# Pathogenic mechanisms in West Nile virus infection

West Nile virus (WNV) is a zoonotic arbovirus, belonging to the genus Flavivirus in the Flaviviridae family. WNV has at least five different lineages. Lineage 1, spread worldwide, is subdivided into clades, 1a and 1b. Clade 1a was isolated in America, Africa, Asia, Europe and the Middle East, and was later divided into A and B sub-clades. Clade 1b, or the Kunjin virus (KUNV), is known to be endemic in Australia and only occasionally affects humans. Lineage 2 can be found in Sub-Saharan Africa, Madagascar, South Africa and in some countries in Europe such as Russia, Hungary, Greece, Italy. WNV lineage 2 from Europe has its origins in Africa and has become endemic in the last two decades. Lineage 3 or Rabensburg virus, was isolated from Culex pipiens in the Czech Republic and South Moravia. Lineage 4 was isolated in the Caucasus region (Russia), from a Dermacentor tick and then from mosquitoes. WNV lineage 5 is considered to be clade 1c of lineage 1 and it was isolated initially in humans and mosquitoes in India. Some have discussed the existence of lineage 6, isolated from Culex pipiens in Spain, and also that of lineage 7, isolated from the Koutango virus in Senegal.

In the neural invasion, there are involved the Toll-like receptors 3, the CCR5 receptor and its ligand, CCL5, pushed by the WNV, with the local migration of the T CD4, CD8, NK1.1 lymphocytes and of the macrophages. The central nervous system invasion is produced from one cell to another, with the appearance of local inflammation, brain edema and encephalitis, especially by affecting the hippocampus, thalamus and temporal region, black substance and cerebellum. It is generally accepted that CNS invasion is the consequence of an inefficient systemic immune response, which allows a major viral replication whereby CNS is affected proportionally to the level and duration of the viremia. Several mechanisms of WNV entry into the CNS have been proposed, for which there is experimental evidence on mice: passive transport through the choroid plexus epithelial cells, through the olfactory neurons or through the infected immune cells. The CNS infection occurs most often in the cortex, cerebellum, basal ganglia and the brain stem, and rarely in the hippocampus or the olfactory bulb. WNV acts primarily at the neuronal level: at the level of microglia, astrocytes, endothelial cells, oligodendrocytes, and neuroblastoma cells but also at a spinal cord level, with the anterior gray column being affected most often.

The emergence of severe cases in immunocompromised persons and in the elderly seems to be explained by the decrease of the immune response to new antigens, which follows

as a result of fewer T cells being produced by the thymus and the ineffective response of neutralizing IgM antibodies. LyCD4+ support a viral clearance response of LyCD8+ in the CNS, the latter being responsible for controlling the infection and preventing viral persistence in the tissue. IgM and IgG antibodies are responsible for controlling the viremia and for preventing a fatal outcome. The role of the microglia, astrocytes and oligodendrocytes (involved in the production of myelin) in the WNV infection has also been studied. Present in a ratio to neurons of 1.4, astrocytes represent the neuronal metabolic support and are essential to brain homeostasis. They play a limited part in the synthesis of acute phase proteins and proinflammatory cytokines but a crucial one in controlling leukocyte influx in the CNS, maintaining the integrity of the Blood-Brain-Barrier (BBB) through the glia limitans network. Microglia represent the CNS macrophages and are activated during inflammatory diseases or neuronal injuries. Their plasticity allows them to modify their cellularity numerically, morphologically and as an expression of surface receptors, as well as to synthesize proinflammatory cytokines and growth factors. After the viremia peaks, WNV enters into the CNS, where leukocyte recruitment takes place through the transmigration of peripheral leukocytes at the level of the BBB and at the vascular endothelium level. The leukocytes are then retained in the perivascular space. Leukocyte migration is influenced by not fully known mechanisms at the moment. The ability of TNF-α to increase vascular permeability is easy to see, as is the expression of the C-X-C motif chemokine 12 (CXCL12) (McCandless EE, 2006), and the effect of matrix metalloproteinases (MMP) produced by the astrocytes, which facilitate the leukocyte migration into the perivascular space and at the level of the glial limitans. The leukocyte migration in the brain parenchyma is the outcome of injuries that occurr at the level of glia limitans components such as collagen degradation under the action of cysteine protease cathepsins K, S, and L, the conversion of plasminogen into plasmin and the fibronectin and laminin degradation. During the CNS invasion of WNV, there is an increase in the expression of two CXC chemokines-type, CXCL1 and CXCL2 that contribute to the neutrophil recruitment in the brain. Neutrophils are the most important immune cells involved in the early local defense. WNV induces neuronal apoptosis through the caspase-3 and caspase-9 pathways, activated by the presence of capsidic WNV proteins in the CNS, but also by the activation of calphain and cathepsin. High levels of WNV structural proteins (NS2A, 2B, 4A, 4B) and E glycoprotein may induce apoptosis via endoplasmic reticulum (ER) stress. Research in the pathogenesis of WNV neuroinvazive infection highlights the hightened viral action that follows the release by neurons of proinflammatory cytokines such as IL-1β, -6, -8 and TNF-α. Differences in virulence among strains of WNV is explained by

the ability of virulent strains – for e.g. WNV-NY99 1999 New York, to act as a potent antagonist of alpha/beta interferon (IFN-α / β), mediated by Janus kinase - signal transducer and activator of transcription (JAK-STAT) by inhibiting JAK phosphorylation, by the intervention of nonstructural proteins NS2A, NS2B, NS3, NS4A, and NS4B. It has also been hypothesised that the insufficiency of down-regulated TLR3 macrophages, present in the elderly, is responsible for the presence of high levels of proinflammatory cytokines with vasculogenic propertie. Among the WNV nonstructural proteins, NS1 plays an antagonist role in antiviral defense by inhibiting complement activation, TLR3 signal transduction and by activating STAT1/STAT2.

The knowledge of pathogenetic mechanisms is extremely important in understanding the evolution of neurological manifestations of the West Nile virus infection and the potential therapeutic management strategies, ideas that I intend to investigate. I will focus my research on changes in the activation of thrombin in West Nile virus encephalitis as a potential predictor factor for an unfavorable evolution.