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Immunity Versus Immunopathology in West Nile Virus Induced Encephalitis

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1. Introduction

West Nile virus (WNV), a mosquito-borne neurotropic pathogen, belongs to the family of Flaviviridae, the genus Flavivirus, a group of plus-sense, single-stranded RNA viruses (Anderson *et al.*, 1999; Lanciotti *et al.*, 1999). WNV genome is a single-stranded, positive-sense RNA molecule, approximately 11,000 nucleotides in length that is translated into a single polypeptide, which is co- and post-translationally processed into ten proteins – three structural proteins (envelope (E), membrane and nucleocapsid) and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Anderson *et al.*, 1999; Lanciotti *et al.*, 1999). The virus was originally isolated in Africa, and later caused epidemics with mainly a febrile illness in humans in Europe, the Middle East, and parts of Asia. In 1999, a more virulent WNV strain was detected in New York City. Since then, it has rapidly spread throughout the continental United States, southern Canada, Mexico, Guatemala, the Caribbean and to several countries in South America. It has become a public health concern in North America over the past decade (Campbell *et al.*, 2002). The virus is maintained in an enzootic cycle that involves mosquitoes and birds. Human infection results primarily from mosquito bites; blood transfusion, organ transplantation, breast feeding and *in utero* or occupational exposure have all been associated with viral infection (2002a; 2002b; Alpert *et al.*, 2003; Charatan, 2002). Although most WNV infections in humans are asymptomatic, severe neurological disease (including encephalitis) and death have been observed with a higher frequency in the elderly and immunocompromised (Campbell *et al.*, 2002; Pletnev *et al.*, 2006). Recent evidence also suggests that WNV can persist for years in humans and animals convalescing from infection (Appler *et al.*, 2010; Murray *et al.*, 2010; Tesh *et al.*, 2005). Currently, licensed vaccines are not yet ready to use in humans. Treatment is currently nonspecific and supportive (Campbell *et al.*, 2002).

WNV has been studied in various animal models, including mice, hamsters, monkeys and horses (Davis *et al.*, 2001; Kramer & Bernard, 2001; Ratterree *et al.*, 2004; Xiao *et al.*,

2001). Following the initial subcutaneous or intraperitoneal infection in mice, WNV induces a systemic infection, invades the central nervous system (CNS) and causes death rapidly when encephalitis develops, usually within 1–2 weeks (Beasley *et al.*, 2002; Kramer & Bernard, 2001; Wang *et al.*, 2001b). The severity and symptoms of lethal infection observed in mice mimic the symptoms caused by WNV infection in humans. The murine model has been an effective *in vivo* experimental model to investigate viral pathogenesis and the host immunity in humans. Based on information obtained from studies on animal models, cell culture and patient samples, this review will be focused on discussion of the role of several important immune factors, including pathogen recognition receptor (PRR) - mediated signaling pathways, cytokines, monocytes/microglia, γδ T cells, $CD4^+$ and $CD8^+$ αβ T cells in protection and pathogenesis of WNV-induced encephalitis.

2. Innate Immunity to WNV infection

2.1 PRR signaling pathways

The key players of the innate immune surveillance are the sensor molecules known as PRRs which recognize specific pathogen associated molecular patterns (PAMPs) and trigger the signaling cascade ultimately leading to the production of type 1 interferon (IFN)s and pro-inflammatory cytokines. Three classes of PRRs have been implicated for viral PAMPs: toll-like receptors (TLRs), retinoid acid-inducible gene-I (RIG-I) -like receptors (RLRs), and nucleotide oligomerization domain (NOD) -like receptors (NLRs) (Iwasaki & Medzhitov, 2010; Wilkins & Gale, 2010). Of these, several TLRs and RLRs are involved in WNV recognition.

2.1.1 TLRs

TLRs, a family of thirteen mammalian homologues of *Drosophila* Toll that recognize PAMPs, play an essential role in the initiation of innate immunity (Qureshi & Medzhitov, 2003). Most TLR signaling pathways (except TLR3) utilize myeloid differentiation factor 88 (MyD88) as the primary adaptor (Akira & Hemmi, 2003). TLR stimulation culminates in the synthesis of antiviral cytokines, such as type 1 IFN and proinflammatory cytokines, which may directly suppress viral replication. WNV is a positive ssRNA virus that produces dsRNA in its life cycle (Samuel, 2002). TLR3 recognizes dsRNA and is expressed in dendritic cells (DCs) and several CNS cell types, including neurons, astrocytes, and microglia (Daffis *et al.*, 2008; Town *et al.*, 2006; Wang *et al.*, 2004). TLRs 7 and 8 are implicated in MyD88-dependent recognition of ssRNA and ssRNA-producing viruses. Depending on the virus dose (lethal versus sub-lethal), passage history of the virus (Vero cell-derived versus insect cell- derived) or routes of inoculation, TLRs 3 and 7 are known to play important roles in host immunity to WNV infection, either pathogenic or protective. Following a sub-lethal dose of insect cell derived WNV infection either intraperitoneally or subcutaneously, TLR3 provides a protective effect against WNV infection, partially by restricting replication in neurons (Daffis *et al.*, 2008). WNV NS1 protein plays a role in viral pathogenesis by counteracting TLR3 signaling in *in vitro* cell culture (Wilson *et al.*, 2008). During an intraperitoneal infection of WNV, TLR7-mediated signaling promoted IL-12/IL-23-dependent immune cell homing to infected target cells, thereby contributing to a vital host defense mechanism (Town *et al.*, 2009). MyD88-mediated signaling was reported to restrict

WNV by inhibiting replication in neurons and modulating expression of chemokines that regulate immune cell migration into the CNS (Szretter *et al.*, 2010).
TLR3 and TLR7 -mediated signaling can also be pathogenic to host during WNV infection. For example, upon a lethal dose of a mammalian cell-passaged WNV challenge in mice via the intraperitoneal route, TLR3-dependent proinflammatory cytokines , including tumor-necrosis factor (TNF)-α were involved in blood brain barrier (BBB) compromise, and neuronal injury (Wang *et al.*, 2004). In young human donors, binding of the glycosylated WNV envelope protein to the C-type lectin DC-specific intercellular adhesion molecule 3 (ICAM3) grabbing nonintegrin (DC-SIGN) leads to a reduction in the expression of TLR3 in macrophages via the signal transducer and activator of transcription 1 (STAT1)-mediated pathway. This signaling was shown to be impaired in the elderly, which led to higher levels of TLR3 and proinflammatory cytokines. Thus, the alteration of the innate immune response with aging may lead to higher TLR3 levels and increased BBB permeability, which may suggest a possible mechanism for the increased severity of WNV infection in older individuals (Kong *et al.*, 2008). Another example, WNV permissive Langerhans cells (LCs) could migrate from the skin to the lymph nodes upon a cutaneous infection, a process that might contribute to WNV dissemination in early viral infection (Byrne *et al.*, 2001; Johnston *et al.*, 2000). TLR7 recognition of WNV in skin epidermal keratinocytes induced IFN-α, interleukin-1β (IL-1β), IL-6 and IL-12 responses were shown to promote LC migration from the skin and WNV dissemination from the skin to other peripheral organs to initiate systemic infection. This effect might compromise its protective effect during a systemic infection (Welte *et al.*, 2009).

2.1.2 RLRs

RLRs, which include RIG-I and melanoma differentiation antigen 5 (MDA-5) recognize dsRNA during viral infection. RIG-I is involved in the initial recognition of WNV infection and controls early virus replication. The wild-type WNV NY99 strain is known to evade the activation of interferon regulatory factor 3 (IRF3) through RIG-I-dependent and -independent pathways without antagonizing host defense signaling (Fredericksen & Gale, 2006). MDA-5 is needed to amplify and maintain the antiviral signals (Fredericksen *et al.*, 2008). Both MDA5 and RIG-I work in concert to maintain the induction of the antiviral genes, while IFN-α functions to amplify and/or expand the response in an attempt to control viral replication. They are responsible for triggering downstream gene expression in response to WNV infection by signaling through an adaptor, interferon promoter stimulator-1 (IPS-1), which leads to activation of transcription of IRFs and NF-κB (Fredericksen *et al.*, 2008). IPS-1 also plays a role to modulate adaptive immune response by providing effective antibody response and by restricting the expansion of regulatory T cells, though the mechanism needs to be deciphered. (Suthar *et al.*, 2010). RIG-I signaling is regulated by multiple host factors, including ubiquitination, autophagy or the RNA helicase LGP2 (Gack *et al.*, 2007; Jounai *et al.*, 2007; Saito *et al.*, 2007). Caspases are a family of aspartic acid–specific cysteine-dependent proteases mainly involved in apoptotic and inflammatory signaling pathways. During WNV infection, Caspase 12 has been found to be required for an effective antiviral innate response by regulating the ubiquitination of RIG-I through the tripartite motif (TRIM) 25 mediated pathway (Wang *et al.*, 2010).

2.2 Cytokines

2.2.1 IFNs

Type 1 IFNs, including IFN-α and IFN-β participate in the control of viral infections (Katze *et al.*, 2002) and *in vitro*, can partially protect fetal murine spinal cord tissues, neuroblastoma cells and primate cells from WNV infection (Anderson & Rahal, 2002; Lucas *et al.*, 2003; Samuel & Diamond, 2005; Shahar *et al.*, 1990). IFN-α/β $R^{-/-}$ mice were much more susceptible to WNV infection than controls (Samuel & Diamond, 2005). The production of type 1 IFNs after flavivirus infection is primarily triggered by viral RNA through several distinct PRRs including the cell surface and endosomal RNA sensors TLR3 and TLR7, and the cytoplasmic RNA sensors, RIG-I and MDA-5 (2002c; Daffis *et al.*, 2008; Fredericksen & Gale, 2006; Fredericksen *et al.*, 2008; Town *et al.*, 2009; Wang *et al.*, 2004). In older donors, the production of type 1 IFNs was significantly lower in DCs, compared with younger donors, which might contribute to their higher susceptibility to WNV encephalitis (Qian *et al.*, 2011). Type 2 IFN, such as IFN-γ was produced by γδ T cells, nature killer (NK) cells and CD8 T cells, which provide protective immunity against lethal WNV encephalitis (Shrestha *et al.*, 2006b; Wang *et al.*, 2003a). Several NS proteins have been reported to be associated with evasion of host innate immune defenses (Laurent-Rolle *et al.*, 2010; Puig-Basagoiti *et al.*, 2007; Rossi *et al.*, 2007), including inhibiting IFN signaling by the blockage of STAT1 and STAT2 activation (Evans & Seeger, 2007; Liu *et al.*, 2005; Munoz-Jordan *et al.*, 2005).

2.2.2 Proinflammatory and regulatory cytokines

Proinflammatory cytokines, such as TNF-α, IFN-γ or IL-1β can act synergistically with WNV to modulate the expression of immune recognition molecules, including class I and II major histocompatibility complex (MHC) and various adhesion molecules on endothelial cell surface, leading to increased recognition by the virus-specific cytotoxic T cells (King *et al.*, 2003). Neuronal TNF-α expression could diminish chemokine (C-X-C motif) ligand 10 (CXCL10) -induced death in the CNS (Zhang *et al.*, 2010a).

TNF-α and IL-1β were up-regulated with replication of the E protein-glycosylated virus indicating a relation to the neuroinvasive phenotype of E protein-glycosylated WNV (Shirato *et al.*, 2006). Microarray analysis of genes upregulated by neurovirulent strains of WNV also revealed the involvement of TNF-α and other inflammatory cytokines in both mouse (Venter *et al.*, 2005) and human cells (Cheeran *et al.*, 2005) following WNV infection. Consistent with these findings, WNV replication induced TNF-α and macrophage migration inhibitory factor (MIF) responses during systemic infection were shown to modulate the BBB permeability, which in turn may enable viral entry into the brain and induce lethal encephalitis (Arjona *et al.*, 2007; Wang *et al.*, 2004). Regulatory cytokines, such as IL-10 is known to be involved in WNV pathogenesis (Bai *et al.*, 2009; Schneider *et al.*, 2007). WNV infection was diminished in IL-10-deficient mice, and this ultimately increased the survival rate (Bai *et al.*, 2009; Schneider *et al.*, 2007). Another regulatory cytokine, transforming growth factor (TGF)-β was found to suppress the protective γδ T cell subsets expansion during WNV infection (Welte T. & Wang T, unpublished data).

2.2.3 Chemokines

Chemokines and chemokine receptors mediate leukocyte trafficking during WNV infection. Cxcr2 is important for early neutrophil migration to the initial site of virus entry (Bai *et al.*,

2010). Ccr2 is critical for monocyte recruitment to the CNS, acting mainly by regulating monocytosis in the blood (Lim *et al.*, 2011). Cxcr3 and Cxcl 10 appear to control $CD4^+$ and $CD8^+$ T lymphocyte accumulation in the brain (Klein *et al.*, 2005; Zhang *et al.*, 2008). CNS expression of the chemokine receptor CCR5 and its ligand CCL5 was prominently up-regulated by WNV, and this was associated with the infiltration of $CD4^+$ and $CD8^+$ T cells, $NK1.1^+$ cells and macrophages expressing the receptor (Glass *et al.*, 2005). In humans, CCR5 may function normally to limit disease due to WNV infection (Lim *et al.*, 2010). It is suggested that CCR5 deficiency is a strong and consistent risk factor for symptomatic WNV infection in the United States (Lim *et al.*, 2008).

2.3 Cellular players: Monocytes /macrophages, microglia, neutrophils, NK cells and γδ T cells

2.3.1 Monocytes /macrophages and microglia

Initial studies suggest that macrophages are important in the non-specific immediate defense system of WNV infection. *In vivo* depletion of macrophages had an exacerbating effect on the course of the infection by an attenuated WNV strain, exhibited by higher and extended viremia and accelerated development of encephalitis and death (Ben-Nathan *et al.*, 1996).

Nevertheless, the susceptibility of monocytes /macrophages to productive WNV infection *in vitro* (Cardosa *et al.*, 1983) is also compatible with a potential role in initial WNV replication and propagation in humans (Rios *et al.*, 2006). In support of this notion, silencing early viral replication in macrophages in mice seems to effectively suppress WNV induced encephalitis (Ye *et al.*, 2003). In the CNS, microglia and macrophage-associated inflammation are involved in neuropathology (Wang *et al.*, 2004). An attenuated microglia activation in *Tlr3*$^{-/-}$ brains was reported to contribute to the resistance of these mice to WNV encephalitis during systemic infection (Wang *et al.*, 2004). Although there were more microglia in the CNS during WNV infection, few of them were proliferating, which suggests that the increased numbers in the CNS might be derived from a migratory precursor cell. Indeed, a recent report shows that $Ly6c^+Gr1^{hi}Ly6C^{hi}CCR2^+$ "inflammatory monocytes" are microglial precursors recruited to the CNS during WNV infection (Getts *et al.*, 2008). Consistent with these findings, CCL2-dependent inflammatory monocyte migration was reported to be critical for increases in microglia during WNV infection and may play a pathogenic role during WNV encephalitis (Lim *et al.*, 2011).

2.3.2 Neutrophils

Neutrophils are the most abundant type of leukocytes in humans, a key component of the innate immune response, and the first immune cells to be recruited to inflammatory foci. Following a lethal dose of WNV challenge, neutrophils greatly expand as the virus invades the brain (Brehin *et al.*, 2008). Neutrophils play a paradoxical role during WNV infection. Depletion of these cells can be either beneficial or harmful during WNV infection, depending on the timing of depletion. Neutrophil depletion 1 or 2 days following infection resulted in increased susceptibility whereas unexpectedly, if neutrophils were depleted 1 day prior to infection, the opposite results were observed: increased resistance (Bai *et al.*, 2010).

2.3.3 NK cells

NK cells are a crucial component of the host innate immune system with anti-viral properties. The role of NK cells in WNV infection is controversial. They are reported to be important to control WNV infection by recognition and elimination of WNV infected cells. Infection of mice with WNV was accompanied by temporary activation of NK cells (Vargin & Semenov, 1986). Interaction of NKp44 with the WNV E protein is an important step in triggering NK cell activation during infection (Hershkovitz *et al.*, 2009). A more recent study shows that co-culture of peripheral blood mononuclear cells with K562D2 stimulatory cells is an efficient technique to prepare large quantities of pure and active human NK cells, and these expanded NK cells inhibited WNV infection of Vero cells through both cytolytic and noncytolytic activities, which may imply a potential role of NK cells in combating WNV infection (Zhang *et al.*, 2010b). Nevertheless, antibody depletion of NK cells in mice did not show enhanced susceptibility to WNV encephalitis (Chung *et al.*, 2007; Shrestha & Diamond, 2004).

2.3.4 γδ T cells

In mice and humans, γδ T cells comprise a minority of the $CD3^+$ T cells in lymphoid tissue and blood but are well represented at epithelial and mucosal sites (Hayday, 2000). They can rapidly produce Th1, Th2, or Th-17 type cytokines dependent upon the type of antigen or the subtype of γδ T cells stimulated (Carding & Egan, 2002; Hayday, 2000; Hayes & Love, 2002; Roark *et al.*, 2007) and have unique features, including a lack of MHC restriction and the potential capacity to respond to antigens without a requirement for conventional antigen processing, which together suggest a role in innate immunity against pathogen infection (Wang *et al.*, 2001a). γδ T cells are important for early control of WNV dissemination (Wang *et al.*, 2003a). $TCR\delta^{-/-}$ mice, which are deficient in γδ T cells had elevated viral loads and greater dissemination of the pathogen to the CNS, more severe encephalitis and thereby were much more susceptible to WNV infection than wild-type controls. This protection relied partially on their IFN-γ producing capacity. Adoptive transfer of wild-type or $TCR\beta^{-/-}$ mice splenocytes to naïve $TCR\delta^{-/-}$ mice enhanced survival, whereas $TCR\beta^{-/-}$ IFN-$\gamma^{-/-}$ splenocoytes did not. Further, irradiated mice reconstituted with IFN-γ-deficient γδ T cells had significantly higher levels of viral loads in blood, and brains throughout the time course compared to mice reconstituted with IFN-γ-sufficient γδ T cells (Shrestha *et al.*, 2006b). γδ T cells are further divisible into functionally distinct subsets in human and mouse, which have direct and indirect effects on host immunity to pathogen infection (Bank *et al.*, 1986). Following WNV challenge, $V\gamma1^+$ cells, the major subpopulation, expanded significantly and were the main resource for IFN-γ. Mice depleted of $V\gamma1^+$ cells had an enhanced viremia and higher mortality to WNV encephalitis. A major risk factor for fatality of WNV infection in humans is aging (Hayes & Gubler, 2006; Wang & Fikrig, 2004). Aged mice were more susceptible to WNV infection than young mice. $V\gamma1^+$ T cells in aged mice displayed a slower and reduced response following WNV infection, which might partially contribute to the enhanced host susceptibility to viral encephalitis (Welte *et al.*, 2008). Human γδ T cells are also known to display numerical and functional alteration in the elderly (Argentati *et al.*, 2002; Cardillo *et al.*, 1993; Weerkamp *et al.*, 2005). Nevertheless, whether the dysfunction of γδ T cells of older individuals leads to their higher susceptibility to WNV encephalitis remains undefined.

Vγ4$^+$ cells, another peripheral γδ T cell subset, had a higher potential for producing TNF-α, a cytokine known to be involved in BBB compromise and WNV entry into the brain. Depletion of Vγ4$^+$ cells reduced TNF-α level in the periphery, accompanied by a decreased viral load in the brain and a lower mortality to WN encephalitis (Welte *et al.*, 2008).

3. DCs, the linkage between innate and adaptive immunity

DCs, macrophages, and B cells are the antigen presenting cells (APCs) involved during WNV infection (Kulkarni *et al.*, 1991). DCs represent the most important APCs exhibiting the unique capacity to initiate primary T cell responses. In particular, during cutaneous WNV infection, the bone-marrow-derived epidermal DCs – LCs are important APCs in the skin — where the pathogen is naturally deposited during mosquito transmission of the virus (Byrne *et al.*, 2001; Johnston *et al.*, 2000). These cells migrate from the epidermis by an IL-1β-dependent pathway and accumulate in the local draining lymph nodes, thereby playing an important role in T-cell activation and proliferation (Byrne *et al.*, 2001). Interestingly, a recent report shows that mosquito saliva contains factors that could alter the antiviral signaling of APCs, including macrophages and DCs, which may explain the enhancement of WNV diseases during a natural infection (Schneider *et al.*, 2010). Upon microbial infection, DC maturation is an innate response that leads to adaptive immunity to foreign antigens (Bennett *et al.*, 1998; De Smedt *et al.*, 1996). Maturation of DCs results in the expression of high levels of MHC and co-stimulatory molecules such as CD40, CD80 and CD86 and is often associated with the secretion of IL-12 (Fujii *et al.*, 2004; Inaba *et al.*, 2000). WNV-induced γδ T-cell activation plays an important role in promoting DC maturation, which further initiates CD4$^+$ T-cell priming. Splenic DCs of WNV-infected TCRδ$^{-/-}$ mice displayed lower levels of CD40, CD80, CD86 and MHC class II expression and IL-12 production than those of wild-type mice (Fang *et al.*, 2010).

WNV permissive LCs could migrate from the skin to the lymph nodes upon a cutaneous infection, which contributes to WNV dissemination in early viral infection (Byrne *et al.*, 2001; Johnston *et al.*, 2000). DC-SIGN is highly expressed in monocyte-derived DCs *in vitro* and at lower levels *in vivo* in subsets of macrophages and DCs (Geijtenbeek *et al.*, 2000; Krutzik *et al.*, 2005; Soilleux *et al.*, 2002). DC-SIGN plays an important role in the enhancement of infection by WNV glycosylated strains. Further, the location of the N-linked glycosylation sites on a virion determines the types of glycans incorporated, thus controlling viral tropism for DC-SIGN -expressing cells (Davis *et al.*, 2006).

4. Adaptive immunity

4.1 T cell responses

Both CD4$^+$ and CD8$^+$ αβ T cells contribute to host survival during WNV infection. CD4$^+$ T cells respond vigorously in the periphery (Kulkarni *et al.*, 1991). They are known to provide help for antibody responses and to sustain WNV-specific CD8$^+$ T cell responses in the CNS enabling viral clearance (Sitati & Diamond, 2006). Among CD4$^+$ αβ T cells, higher levels of peripheral Tregs after infection protect against severe WNV disease in immunocompetent animals and humans possibly by dampening the WNV-specific immune response and inflammation. In humans, WNV symptomatic donors exhibited lower Treg frequencies from 2 weeks through 1 year after index donation. Similarly, symptomatic WNV-infected mice

also had lower Treg frequencies compared with asymptomatic mice at 2 weeks after infection (Lanteri *et al.*, 2009). In WNV-infected mice, $CD8^+$ T cell responses have been observed in both the spleen and brain (Liu *et al.*, 1989). $CD8^+$ T cells have important functions in clearing infection from peripheral tissues and CNS, and in preventing viral persistence (Brien *et al.*, 2007; Shrestha & Diamond, 2004). During WNV infection, chemokines secreted from CNS, including CXCL10 (Klein *et al.*, 2005) or CCL5 (Glass *et al.*, 2005) help to recruit the antigen specific $CD8^+$ effector T cells. CD40-CD40L interactions are also important for T cell trafficking into the CNS and for protection of the host from a low dose WNV challenge (Sitati *et al.*, 2007). Once inside CNS, these cells kill the virus infected target cells in perforin or FasL effector-dependent manners (Shrestha & Diamond, 2007; Shrestha *et al.*, 2006a). Both primary and memory $CD8^+$ T cells have been demonstrated to efficiently kill target cells that display WNV antigens restricted by a class I MHC (Shrestha & Diamond, 2004).

αβ T cells can also be pathogenic. For example, they can support a low but productive WNV replication. Following a systemic infection, WNV might cross the BBB and enter the CNS by being carried by infected infiltrating T cells (Wang *et al.*, 2008). Another study suggests that $CD8^+$ T cells contribute to immunopathology upon high-dose WNV challenge (Wang *et al.*, 2003b). Nevertheless, little is known about the role of T cell-mediated pathology in WNV-related brain damage. In the aged mouse model of WNV infection, defects in T cell responses against dominant WNV epitopes were shown to contribute to the enhanced susceptibility to WNV encephalitis (Brien *et al.*, 2009). Primary and memory T cell responses in old mice induced by RepliVAX WN, a single-cycle flavivirus vaccination were significantly lower than those seen in younger mice. However, this seems to be overcome by repeating *in vivo* stimulation of T cell responses in old mice (Uhrlaub *et al.*, 2011). In investigation of the correlation between T cell phenotype and disease severity, CD8 T cells of a terminally differentiated/cytolytic profile were found to be associated with neuroinvasion (Piazza *et al.*, 2010).

4.2 B cells, antibody and complement responses to WNV infection

B cells and specific antibodies are critical in the control of disseminated WNV infection, but are not sufficient to eliminate it from the host (Diamond *et al.*, 2003a; Diamond *et al.*, 2003b; Diamond *et al.*, 2003c; Roehrig *et al.*, 2001). In particular, induction of a specific, neutralizing IgM response early during infection limits viremia and dissemination into the CNS and protects the host against lethal infection (Diamond *et al.*, 2003c). Mice deficient in B cells and antibody (microMT mice) were vulnerable to lethal WNV infection (Engle & Diamond, 2003). Patients with defects in humoral immunity may not produce a serologic response and clear WNV infection permanently, which would result in persistent CNS infection (Penn *et al.*, 2006).

The complement system is made up of a complex pathway of more than 30 serum proteins and cell surface receptors that are involved in direct cell lysis and the enhancement of B and T cell responses (Avirutnan *et al.*, 2008; Carroll, 2004). Complement is activated by three different pathways: classical, lectin and alternative. All three share the common step of activating the central component C3, but they differ according to the nature of recognition. Although one early study suggests that complement could enhance IgM-dependent WNV replication in macrophages *in vitro* (Cardosa *et al.*, 1983), more evidence supports that it is required for control of WNV infection *in vivo*. Protection against WNV encephalitis requires

an intact complement system as mice lacking C3 uniformly succumbed to infection (Mehlhop *et al.*, 2005). All complement activation pathways are required, as mice deficient in C1q (classical pathway) or fB (alternative pathway) (Mehlhop & Diamond, 2006), or mice deficient in lectin pathway recognition molecules (mannose binding lectin-A and mannose binding lectin-C or the effector enzyme mannan-binding lectin-associated serine protease-2) were more vulnerable to WNV infection (Fuchs *et al.*, 2011). The complement system is known to control WNV infection, in part through its ability to induce a protective antibody response and by priming adaptive immune responses through distinct mechanisms (Mehlhop & Diamond, 2006; Mehlhop *et al.*, 2005). Mice deficient of classical and lectin pathways had defects in WNV specific antibody production and T cell responses. In comparison, mice deficient of alternative pathway had normal B cell function but impaired $CD8^+$ T cell response (Mehlhop & Diamond, 2006).

5. Conclusions

Studies from animal models, cell culture and patient samples have suggested that both innate and adaptive immunity are involved in host protective immune responses. Among them, type 1 IFNs, γδ T cells and humoral immunity are critical in controlling dissemination of WNV (Anderson & Rahal, 2002; Diamond *et al.*, 2003a; Fredericksen *et al.*, 2008; Klein *et al.*, 2005; Lucas *et al.*, 2003; Roehrig *et al.*, 2001; Wang *et al.*, 2003a). $CD4^+$ (Kulkarni *et al.*, 1991) and $CD8^+$ αβ T-cells (Shrestha *et al.*, 2006a; Wang *et al.*, 2003b) contribute to host survival following WNV infection. Host immune responses may act as a double-edged sword during WNV infection. Depending on the virus dose (lethal versus sub-lethal), passage history of the virus (Vero cell-derived versus insect cell derived) or routes of inoculation, immune factors can be either pathogenic or protective in host immunity against WNV infection.

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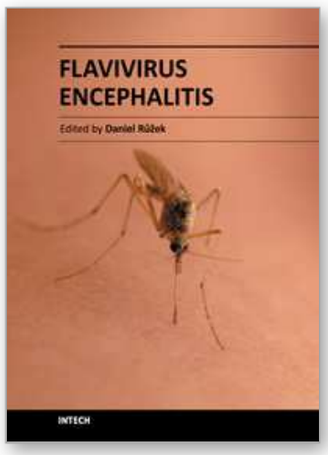

Flavivirus Encephalitis

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Encephalitis is an inflammation of the brain tissue associated with clinical evidence of brain dysfunction. The disease is of high public health importance worldwide due to its high morbidity and mortality. Flaviviruses, such as tick-borne encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, or St. Louis encephalitis virus, represent important causative agents of encephalitis in humans in various parts of the world. The book Flavivirus Encephalitis provides the most recent information about selected aspects associated with encephalitic flaviviruses. The book contains chapters that cover a wide spectrum of subjects including flavivirus biology, virus-host interactions, role of vectors in disease epidemiology, neurological dengue, and West Nile encephalitis. Special attention is paid to tick-borne encephalitis and Japanese encephalitis viruses. The book uniquely combines up-to-date reviews with cutting-edge original research data, and provides a condensed source of information for clinicians, virologists, pathologists, immunologists, as well as for students of medicine or life sciences.

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