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Review

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Encephalitis caused by flaviviruses

L. TURTLE, M.J. GRIFFITHS and T. SOLOMON

From the Department of Infection, Microbiology and Immunology, University of Liverpool, 8th Floor, Duncan Building, Daulby Street, Liverpool, UK

Address correspondence to L. Turtle, Institute of Infection & Global Health, The University of Liverpool, The Apex Building, 8 West Derby Street, Liverpool L69 7BE, UK. email: lturtle@liverpool.ac.uk

Summary

The genus *Flavivirus*, family *Flaviviridae*, contains some of the most important arboviral pathogens of man. The genus includes several aetiological agents of encephalitis, the most significant being Japanese encephalitis virus, West Nile virus and tick-borne encephalitis virus. In each case, the majority of exposed individuals will not develop disease, but a minority will develop a severe illness with a significant chance of permanent neurological damage or death. The factors that determine this are numerous, involving complex interactions between virus and host and are still being actively uncovered. In many cases it appears that the immune response, while crucial to containing the virus and limiting spread to the brain, is also responsible for causing neurological damage. Innate responses can limit viral replication but may also be responsible for generating pathological levels of inflammation. Neutralizing antibody responses are protective but take time to develop. The role of T cells is less clear, and may be either protective or pathogenic. This review summarizes recent developments in the understanding of the pathogenesis of encephalitis caused by flaviviruses.

Although there are about 70 members of the genus *Flavivirus* (family *Flaviviridae*), the most important causes of encephalitis are Japanese encephalitis virus (JEV), West Nile virus (WNV) and Tick-borne encephalitis virus (TBEV). The genus is named after yellow fever virus (in Latin, yellow is flavus), and also includes dengue viruses, which cause fever and rash, as well as St Louis encephalitis virus (SLEV) and Murray Valley encephalitis virus (MVEV).

This review will briefly consider the epidemiology and clinical features of flavivirus encephalitis before examining recent advances in the immunology and pathogenesis; although flaviviruses cause encephalitis on five continents, the review will focus on JEV, WNV and TBEV, drawing on recent data from clinical studies and mouse models.

Epidemiology and clinical features

Since they are transmitted by arthropods (insects or ticks) these viruses are known as arboviruses (arthropod-borne viruses), and except for dengue, they are all zoonotic (i.e. animal viruses that spill over into humans). JEV, WNV, SLEV and MVEV are transmitted principally among birds by *Culex* mosquitoes that breed in muddy water. Although they are genetically closely related viruses, they are found in geographically different parts of the globe; however, they are tending to spread and can cause unexpected outbreaks. JEV occurs in the Asia-Pacific region, MVEV in Australia and nearby islands and SLEV is confined to the Americas[1]; WNV was found in Africa and the Middle East, but in recent years has caused outbreaks in Southern

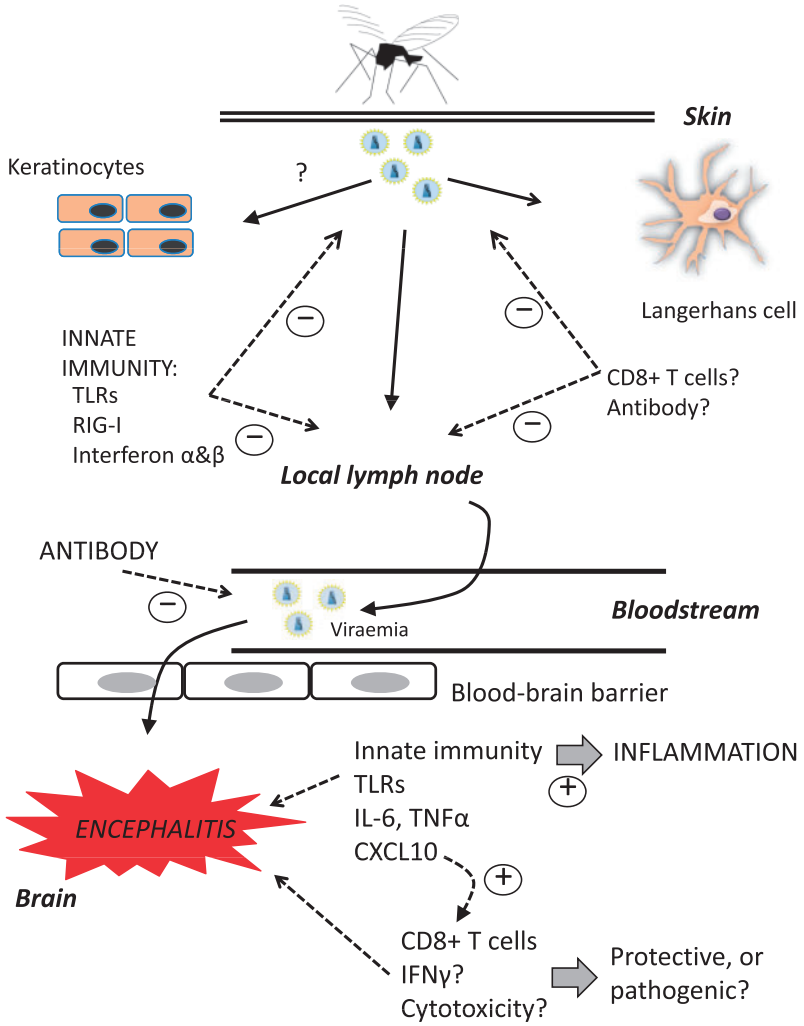

Figure 1. Pathogenesis of flavivirus encephalitis. Key mechanisms in immune control or immunopathology of flavivirus infection are shown in capitals. TLR, toll-like receptor; IL-6, interleukin 6; TNF, tumour necrosis factor; CXCL, C-X-C motif-containing chemokine ligand.

Europe, and reached America, where it rapidly spread across the continent.[2] TBEV has quite different ecology and epidemiology. It is transmitted between small mammals in the forests of central Europe and Russia by hard (ixodid) ticks.

Many infections with these viruses are asymptomatic, or cause a non-specific febrile illness. Neurological presentations include aseptic meningitis, febrile convulsions in children, encephalitis or myelitis—which may cause a polio-like flaccid paralysis. Since it is more abundant, JEV tends to infect children, and so most disease is in this group; WNV, SLEV and MVEV are more likely to cause disease in adults.

Pathogenesis

The pathogenesis of flavivirus encephalitis remains incompletely understood. Aspects of viral replication, central nervous system (CNS) invasion and the nature of the immune response are still being uncovered. Flaviviruses are small single-stranded, positive-sense enveloped RNA viruses. The degree of sequence diversity seen in flaviviruses is less than that observed in other RNA viruses that establish chronic infection, such as HIV and hepatitis C virus. Although viral variation does play an important role in flavivirus pathogenesis, the host immune response is probably a more critical determinant of clinical outcome, at least in humans. An overview of the pathogenesis of flavivirus encephalitis is presented in Figure 1.

Viral entry and replication

Following inoculation from the bite of an infected mosquito, virus is thought to undergo replication in the local tissues mainly in Langerhans cells. For

WNV, replication in keratinocytes, which gives a much larger target cell reservoir, has also been shown.[3] Following replication, virus disseminates to the local lymph nodes where further replication in monocyte lineage cells takes place, causing a viraemia that is followed later by spreading to the brain. In mice, high rates of JEV replication within dendritic cells are linked to higher overall mortality[4] and stopping early viral replication in monocyte-lineage cells using small-interfering RNAs prevent development of WNV and SLEV encephalitis.[5] Interestingly, insertion of brain-specific microRNA complementary sequences into flavivirus genomes abolished virulence suggesting similar technology can in principle work in the brain as well.[6] Autophagy (internal phagocytosis for recycling cell components), which is known to be important in dengue virus and HCV replication, is also involved in early JEV replication. Enhancement of autophagy increased JEV replication and inhibition reduced JEV replication.[7]

How the neurotropic flaviviruses gain access to the CNS remains incompletely understood, but interactions at the blood–brain barrier (BBB) are probably critical. Proposed mechanisms include active replication within endothelial cells, passive transfer across the BBB or within leucocytes that migrate across the barrier.

Innate immunity

Flaviviruses interact with several host cytosolic pathogen-recognition receptors that detect RNA viruses, such as toll-like receptor (TLR)3, TLR7 and the retinoic acid-inducible gene (RIG)-I. For WNV, TLR3 and intracellular adhesion molecule (ICAM)1 (an adhesion molecule found on the vascular endothelium) have been implicated in viral CNS entry through increasing BBB permeability,[8,9] though the detrimental role of TLR3 in mice has been questioned more recently.[10] In human primary macrophages from young donors TLR3 is downregulated upon infection with WNV, but not in elderly donors (in which age group WNV infection is more commonly fatal).[11] The decrease in TLR3 expression resulted in turn in reduced production of pro-inflammatory cytokines in younger donors, consistent with the inflammatory BBB breakdown model of CNS entry in human flavivirus encephalitis. In monocyte-derived dendritic cells the opposite is true, however; younger donors exhibit higher TLR3 expression in response to WNV infection and greater type I interferon (IFN) production.[12] Furthermore, in the case of JEV, for many years clinical and pathological studies have suggested that co-infection with neurocysticercosis (which compromises the BBB), increasing the risk of developing encephalitis following infection with the virus.[13]

After infection is established, neurotropic flaviviruses interfere with innate immunity at a number of levels. Flaviviruses are well known to interfere with IFN antiviral pathways. More recently, WNV was shown to inhibit complement activation by at least two mechanisms, both in solution[14] and on the cell surface.[15] Several genes are known to influence immunity to flaviviruses in animal models. Recently, evidence has accumulated that several of these genes also predispose to disease in humans. 2′–5′ oligoadenylate synthetases (2′–5′ OAS) promote degradation of human and viral RNA. Single-nucleotide polymorphisms (SNPs) in genes encoding for these proteins are associated with susceptibility to infection with WNV in humans[16] (although SNPs in *OAS1*, not *OASL* as originally reported, appear to be functionally important).[17] *OAS1* SNPs are also associated with susceptibility to WNV disease in horses (which have a similar *OAS* gene cluster and response to WNV as humans).[18] More recently, *OAS2* and *OAS3* from the same gene family have been implicated in susceptibility to TBEV disease in humans.[19] In parallel to the findings *in vitro* and in animal models described for WNV and TLR3 above, a functional *TLR3* gene has been associated with increased susceptibility to TBEV infection,[20] suggesting that in humans, overall the role of TLR3 may be pathogenic.

Inflammatory changes

Once in the CNS, there are many mechanisms by which flaviviruses induce neuronal damage and cell death leading to the clinical manifestations of disease. Early histological studies showed that there is a striking inflammatory response in the brain of humans and animals that have died of flavivirus encephalitis. There is typically perivascular inflammation of lymphocytes and macrophages, with glial cell upregulation. In humans with JE, but not in animal models, there are punched out necrotic lesions, often close to blood vessels.[21] In JEV infection, autoimmune demyelination may also occur.[22]

How much brain damage is due to virus-induced cell death, how much is due to (protective?) apoptosis and how much is due to pathological inflammation are not clear. In the case of WNV there appears to be a fine balance whereby immune factors can mediate viral clearance[23–25] but also damage within the CNS.[26,27] JEV-infected mouse peripheral macrophages release mediators that induce apoptosis of uninfected neurons *in vitro*.[28]

Attenuating the CNS inflammatory response to JEV in mice with minocycline results in improved survival.[29,30]

Critical components of adaptive immunity

In recent years, several key experiments in animal models of flavivirus encephalitis have highlighted some of the key components of adaptive immunity that result in virus clearance. It has been known for many years from passive immunization experiments that antibody protects from flavivirus encephalitis in mice. Not surprisingly, therefore, when such experiments were repeated using B-cell-deficient mice the animals were highly susceptible to WNV and JEV infection.[31,32] The role of T cells in flavivirus encephalitis is less clear. This is in part due to variation between different viruses and in the dose, route of administration, mouse strain and age of the mice. In WNV infection $CD8^+$ T-cell knockout mice have increased lethality and viral burden.[23] For JEV, adoptive transfer of cells from immune mice can be clearly shown to be protective in several models, although for optimal protection all components of the immune system should be present.[32] Further evidence for a cooperative function of adaptive immune components was shown by the role of Th2-type CD4 T cells in protection from JEV through help for antibody responses.[33] However, some observations suggest an important role for $CD8^+$ T cells as well. The dominant cell type accumulating in the CNS of JEV-infected mice is a $CD8^+$ T cell.[32] The T-cell receptor usage is highly clonal suggesting that this happens in an antigen-driven manner[34]; recently this has been shown to be the case in WNV and TBEV as well.[35] Moreover, JEV is able to broadly inhibit $CD8^+$ T-cell responses by interfering with antigen presentation, an effect reversible by the addition of TLR3, four or nine ligands.[36] There is also a modest degree of protection seen when immune $CD8^+$ T cells are transferred alone into naïve mice,[32] and though the protective effect of $CD8^+$ T cells is more marked in the case of WNV[23], $CD8^+$ T cells can also be pathogenic under some circumstances in WNV infection.[27] Together, these data suggest that there may be a dual role of $CD8^+$ T cells in both clearing viral infection but also mediating immunopathology within the CNS. Particularly interesting in this regard is the implication that $CD8^+$ T cells provide greater protection from WNV than from JEV, given that JEV is the more pathogenic virus for humans.

Conclusion

In conclusion, flaviviruses are important causes of encephalitis across the globe, and they are spreading. Recent studies on the pathogenesis have shown critical involvement of both innate and adaptive immune responses. However, for both types of response there is evidence for protective and detrimental elements. A major future research question will be to understand the precise balance between immune protection and immunopathology. This will be crucial in developing new, more targeted immunomodulatory therapies for established encephalitis.

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References

1. Solomon T, Dung NM, Kneen R, Gainsborough M, Vaughn DW, Khanh VT. Japanese encephalitis. *J Neurol Neurosurg Psychiatry* 2000; **68**:405–15.
2. Kramer LD, Li J, Shi P-Y. West Nile virus. *Lancet Neurol* 2007; **6**:171–81.
3. Lim PY, Behr MJ, Chadwick CM, Shi PY, Bernard KA. Keratinocytes are cell targets of West Nile virus in vivo. *J Virol* 2011; **85**:5197–201.
4. Wang K, Deubel V. Mice with different susceptibility to Japanese encephalitis virus infection show selective neutralizing antibody response and myeloid cell infectivity. *PLoS One* 2011; **6**:e24744.
5. Ye C, Abraham S, Wu H, Shankar P, Manjunath N. Silencing early viral replication in macrophages and dendritic cells effectively suppresses flavivirus encephalitis. *PLoS One* 2011; **6**:e17889.
6. Heiss B, Maximova O, Pletnev A. Insertion of microRNA targets into the flavivirus genome alters its highly neurovirulent phenotype. *J Virol* 2011; **85**:1464.
7. Li J-K, Liang J-J, Liao C-L, Lin Y-L. Autophagy is involved in the early step of Japanese encephalitis virus infection. *Microbes Infect* 2012; **14**:159–68.
8. Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat Med* 2004; **10**:1366–73.
9. Dai J, Wang P, Bai F, Town T, Fikrig E. ICAM-1 participates in the entry of West Nile virus into the central nervous system. *J Virol* 2008; **82**:4164–68.
10. Daffis S, Samuel MA, Suthar MS, Gale M, Diamond MS. Toll-like receptor 3 has a protective role against West Nile virus infection. *J Virol* 2008; **82**:10349–58.

11. Kong K-F, Delroux K, Wang X, Qian F, Arjona A, Malawista SE, *et al.* Dysregulation of TLR3 impairs the innate immune response to West Nile virus in the elderly. *J Virol* 2008; **82**:7613–23.
12. Qian F, Wang X, Zhang L, Lin A, Zhao H, Fikrig E, *et al.* Impaired interferon signaling in dendritic cells from older donors infected in vitro with West Nile virus. *J Infect Dis* 2011; **203**:1415–24.
13. Desai A, Shankar SK, Jayakumar PN, Chandramuki A, Gourie-Devi M, Ravikumar BV, *et al.* Co-existence of cerebral cysticercosis with Japanese encephalitis: a prognostic modulator. *Epidemiol Infect* 1997; **118**:165–71.
14. Avirutnan P, Fuchs A, Hauhart RE, Somnuke P, Youn S, Diamond MS, *et al.* Antagonism of the complement component C4 by flavivirus nonstructural protein NS1. *J Exp Med* 2010; **207**:793–806.
15. Avirutnan P, Hauhart RE, Somnuke P, Blom AM, Diamond MS, Atkinson JP. Binding of flavivirus nonstructural protein NS1 to C4b binding protein modulates complement activation. *J Immunol* 2011; **187**:424–33.
16. Lim JK, Lisco A, Mcdermott DH, Huynh L, Ward JM, Johnson B, *et al.* Genetic variation in OAS1 is a risk factor for initial infection with West Nile virus in man. *PLoS Pathog* 2009; **5**:e1000321.
17. Yakub I, Lillibridge KM, Moran A, Gonzalez OY, Belmont J, Gibbs RA, *et al.* Single nucleotide polymorphisms in genes for 2′′-5-′′oligoadenylate synthetase and RNase L inpatients hospitalized with West Nile virus infection. *J Infect Dis* 2005; **192**:1741–8.
18. Rios JJ, Fleming JGW, Bryant UK, Carter CN, Huber JC, Long MT, *et al.* OAS1 polymorphisms are associated with susceptibility to West Nile encephalitis in horses. *PLoS One* 2010; **5**:e10537.
19. Barkhash AV, Perelygin AA, Babenko VN, Myasnikova NG, Pilipenko PI, Romaschenko AG, *et al.* Variability in the 2′′-5-′′oligoadenylate synthetase gene cluster is associated with human predisposition to tick-borne encephalitis virus-induced disease. *J Infect Dis* 2010; **202**:1813–8.
20. Kindberg E, Vene S, Mickiene A, Lundkvist A, Lindquist L, Svensson L. A functional toll-like receptor 3 gene (TLR3) may be a risk factor for tick-borne encephalitis virus (TBEV) infection. *J Infect Dis* 2011; **203**:523–8.
21. German AC, Myint KSA, Mai NTH, Pomeroy I, Phu NH, Tzartos J, *et al.* A preliminary neuropathological study of Japanese encephalitis in humans and a mouse model. *Trans R Soc Trop Med Hyg* 2006; **100**:1135–45.
22. Tseng Y-F, Wang C-C, Liao S-K, Chuang C-K, Chen W-J. Autoimmunity-related demyelination in infection by Japanese encephalitis virus. *J Biomed Sci* 2011; **18**:20.
23. Shrestha B, Diamond MS. Role of CD8+ T cells in control of West Nile virus infection. *J Virol* 2004; **78**:8312–21.
24. Klein RS, Lin E, Zhang B, Luster AD, Tollett J, Samuel MA, *et al.* Neuronal CXCL10 directs CD8+ T-cell recruitment and control of West Nile virus encephalitis. *J Virol* 2005; **79**:11457–66.
25. Zhang B, Chan YK, Lu B, Diamond MS, Klein RS. CXCR3 mediates region-specific antiviral T cell trafficking within the central nervous system during West Nile virus encephalitis. *J Immunol* 2008; **180**:2641–9.
26. Zhang B, Patel J, Croyle M, Diamond MS, Klein RS. TNF-alpha-dependent regulation of CXCR3 expression modulates neuronal survival during West Nile virus encephalitis. *J Neuroimmunol* 2010; **224**:28–38.
27. Wang Y, Lobigs M, Lee E, Müllbacher A. CD8+ T cells mediate recovery and immunopathology in West Nile virus encephalitis. *J Virol* 2003; **77**:13323–34.
28. Nazmi A, Dutta K, Das S, Basu A. Japanese encephalitis virus-infected macrophages induce neuronal death. *J Neuroimmune Pharmacol* 2011; **6**:420–33.
29. Mishra MK, Basu A. Minocycline neuroprotects, reduces microglial activation, inhibits caspase 3 induction, and viral replication following Japanese encephalitis. *J. Neurochem* 2008; **105**:1582–95.
30. Das S, Dutta K, Kumawat KL, Ghoshal A, Adhya D, Basu A. Abrogated inflammatory response promotes neurogenesis in a murine model of Japanese encephalitis. *PLoS One* 2011; **6**:e17225.
31. Diamond MS, Shrestha B, Marri A, Mahan D, Engle M. B cells and antibody play critical roles in the immediate defense of disseminated infection by West Nile encephalitis virus. *J Virol* 2003; **77**:2578–86.
32. Larena M, Regner M, Lee E, Lobigs M. Pivotal role of antibody and subsidiary contribution of CD8+ T cells to recovery from infection in a murine model of Japanese encephalitis. *J Virol* 2011; **85**:5446–55.
33. Biswas SM, Ayachit VM, Sapkal GN, Mahamuni SA, Gore MM. Japanese encephalitis virus produces a CD4+ Th2 response and associated immunoprotection in an adoptive-transfer murine model. *J Gen Virol* 2009; **90**:818–26.
34. Fujii Y, Kitaura K, Nakamichi K, Takasaki T, Suzuki R, Kurane I. Accumulation of T-cells with selected T-cell receptors in the brains of Japanese encephalitis virus-infected mice. *Jpn J Infect Dis* 2008; **61**:40–8.
35. Kitaura K, Fujii Y, Hayasaka D, Matsutani T, Shirai K, Nagata N, *et al.* High clonality of virus-specific T lymphocytes defined by TCR usage in the brains of mice infected with West Nile virus. *J Immunol* 2011; **187**:3919–30.
36. Aleyas AG, Han YW, George JA, Kim B, Kim K, Lee C-K, *et al.* Multifront assault on antigen presentation by Japanese encephalitis virus subverts CD8+ T cell responses. *J Immunol* 2010; **185**:1429–41.