

# Interactions of host's innate and adaptive immune components in the pathogenesis of viral encephalitis: a review

## Abstract

Viral encephalitis has attracted the attention of researchers and physicians for millennia. In almost all cases, specific causes of central nervous system (CNS) syndromes of viral origin have been difficult and sometime impossible to identify. Immunological mechanisms involved in viral encephalitis are also diverse and complex which greatly limit the advent of modern antiviral therapies against viral encephalitis. This review will focus on the immunopathogenesis of viral encephalitis. Although a considerable emphasis will be placed on Japanese encephalitis, other viral infections of the CNS will be discussed. Role of Treg and Th17 cells, their plasticity and balance in viral encephalitis will be discussed with great details.

**Keywords:** virus, encephalitis, pathogenesis, innate immunity, adaptive immunity

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**Abbreviations:** CNS, central nervous system; JEV, Japanese encephalitis virus; PAMPs, pathogen associated molecular patterns; LRRs, leucine rich repeats; TIR, toll/il-1 receptor; TM, transmembrane; MV, measles virus; HCMV, human cytomegalovirus; HCV, hepatitis c virus; dsRNA, double stranded RNA; LPS, lipopolysaccharide; LBP, lps-binding protein; MMTV, mouse mammary tumor virus; HPV, human papillomavirus; TIRAP, tir-domain containing adapter protein; IRAK-4, il-1 receptor-associated kinase-4; TRAF6, tnf receptor-associated factor 6; MAPKs, mitogen activated protein kinases; TRAM, (TRIF)/TIR domain-containing adaptor molecule; APCs, antigen-presenting cells; SLE, systemic lupus erythematosus; MS, multiple sclerosis; LCMV, lymphocytic choriomeningitis virus; CSFV, classical swine fever virus; DV, dengue virus; YFV, yellow fever virus

## Introduction

Encephalitis is “the presence of an inflammatory process of the brain associated with clinical evidence of neurologic dysfunction” as defined by the Infectious Diseases Society of America.<sup>1</sup> Though more than one hundred different infectious agents have been identified to cause encephalitis, viral infections are responsible for the majority of cases of infectious encephalitis. Viral infections in the central nervous system (CNS) can alter homeostasis, induce neurological dysfunction and result in serious, potentially life-threatening inflammatory diseases. Viruses that can cause encephalitis include rabies virus, herpes simplex virus, enteroviruses including polioviruses, coxsackieviruses, echoviruses and a number of arboviruses (arthropod-borne viruses). Flavivirus genus contains the most important and widespread arboviruses associated with encephalitis and Japanese encephalitis virus (JEV) is one of them which alone cause some 30,000 to 50,000 cases of encephalitis in Southeast Asia annually.<sup>2</sup> JEV infection causes serious inflammation of the brain, which may lead to permanent brain damage.

In healthy individuals, lymphocyte traffic into the CNS is very low and tightly controlled by the highly specialized blood-brain barrier.

However, neurotropic viruses use some strategies to cross the barrier systems of the CNS. The restricted expression of MHC antigens and the nonrenewable nature of the neuronal cells offer additional challenges to the immune system to detect and to respond to viral infections of the CNS. Therefore, the central nervous system offers a unique organ system in which to study viral immunopathogenesis.

TLRs are transmembrane proteins that recognize and respond to pathogen associated molecular patterns (PAMPs) of microbial pathogens and thus, play crucial role in inducing innate immune response to the pathogens. TLR activation leads to the production of different innate cytokines,<sup>3</sup> more importantly type I IFNs in viral infection which are important components of the host defense against viruses. The cytokines produced by TLR activation determines the nature and magnitude of adaptive immunity, thereby, act as a bridge between innate and adaptive immunity. However, by the virtue of their intracellular life cycle and the complexity of viral proteins, viruses can interact with many different TLRs and thus can evolve a number of ways to evade TLR-specific host responses.

Th1 type CD4<sup>+</sup> T cells play pivotal roles in viral encephalitis through producing cytokines with antiviral functions such as IFN- $\gamma$  and antagonizing the development of Th17 cells.<sup>4</sup> However, they may also play pathogenic role in viral encephalitis, rather than their protective functions which depend on the proinflammatory cytokines produced during host innate responses.<sup>5</sup> IL-17- producing Th17 cells are apparently involved in inflammatory tissue damage, leading to the pathogenesis of various autoimmune diseases. Recently, the production of IL-17 has also been reported in several viral infections which indicate their involvement in viral pathogenesis.<sup>6-8</sup> In some viral infections (eg. Persistent viral infections) Th17 cells are preferentially generated which promote viral persistence by inhibiting apoptosis of infected cells.<sup>9</sup> Treg, a subset of CD4<sup>+</sup> T cells, play pivotal roles in maintaining immune homeostasis and preventing tissue damage by suppressing pro-inflammatory responses. However, in some viral infections CD4<sup>+</sup> Foxp3<sup>+</sup> regulatory T cells (Treg) are preferentially expanded as a mechanism of immune evasion which suppress immune response and thereby, adversely affect virus clearance.<sup>10</sup>

The differentiation of both Th17 and Treg cells are TGF- $\beta$ -dependent, but additional coordinate signaling by IL-6, produced by activated dendritic cells, is critically important in Th17 differentiation.<sup>11,12</sup> Therefore, IL-6 produced during innate response may play important role in balancing Th17 and Treg cells which is essential in the development of balanced immune response. This review will provide valuable insight in to the interactions of host's innate and adaptive immune components in the pathogenesis of viral encephalitis.

## Viral encephalitis with special emphasis on Japanese encephalitis

Viral encephalitis in human causes substantial morbidity and mortality worldwide, frequently resulting in severe neurological sequelae and long-term cognitive impairment, therefore, has attracted the attention of historians and physicians for millennia. Many viral pathogens cause CNS infections in humans. Moreover, new viral infections of the nervous system have been appearing with great regularity.<sup>13</sup> Viruses generally gain access to the CNS by one of two routes, hematogenous or neuronal.<sup>14,15</sup> Hematogenous spread is the most common route which can result in an altered blood-brain barrier,<sup>16</sup> as occurs with arthropod-borne flaviviruses like Japanese encephalitis virus, Murray Valley encephalitis virus and West Nile virus. Alternatively, viruses can enter the nervous system by peripheral intraneuronal routes, as exemplified by herpes simplex virus, rabies virus etc.<sup>17,18</sup>

Japanese encephalitis is still considered as the single most important cause of acute viral encephalitis worldwide, accounting for 30,000 to 50,000 cases and 10,000 to 15,000 deaths annually.<sup>19</sup> Japanese encephalitis virus (JEV) causes serious inflammation of the brain, which may lead to permanent brain damage. Transmission occurs primarily via *Culex tritaeniorhynchus* and *Culex vishnui* mosquitoes. Water birds, including herons and egrets, serve as natural reservoirs and domestic pigs as an important amplifying host.<sup>19</sup> Infected humans are dead-end hosts as the level of viremia and its generally short duration make mosquito infection unlikely. After JE was first identified in Japan in the 1870s, it spread gradually but progressively to the Korean peninsula (1933), the Chinese mainland (1940), the Philippines (1950), Singapore and Malaysia (1952), India (1955), and Southeast Asia (Cambodia, Thailand) (1964-1965). More disturbing has been the recent extension within the Indian subcontinent (Bangladesh, 1977; Pakistan, 1983) and the recent reports of cases of JE from Papua New Guinea and the Torres Strait in Northern Australia (1995).

## Toll-like receptors and viruses

TLRs were first described in *Drosophila* on the basis of their homology to the protein Toll. A search for homologous proteins in mammals revealed the TLRs. TLRs are Type I transmembrane pattern recognition proteins having a variable number of N-terminal leucine rich repeats (LRRs) followed by a cysteine rich domain, a transmembrane (TM) domain, and an intracellular Toll/IL-1 receptor (TIR) domain. TLRs are crucial in the innate immune response to microbial pathogens, in that they recognize and respond to pathogen associated molecular patterns (PAMPs), which lead to activation of intracellular signaling pathways and altered gene expression. In mammals, TLRs function as intermediates by interacting with products of infectious agents and then transmitting signals to a cascade of adapters and kinases that ultimately lead to the activation of transcription of cytokine genes.<sup>3</sup> These cytokines, in turn, activate

cells of the innate immune system (eg. Macrophages, NK cells and neutrophils) and finally stimulate the adaptive immune system.

Intracellular and extracellular TLRs can recognize a wide range of viruses leading to the production of different cytokines, more importantly type I IFNs which are important components of host defense against viruses.<sup>20</sup> On the other hand, by virtue of their intracellular life cycle, viruses can interact with many different TLRs and thus can evolve a number of ways to evade TLR-specific host responses.<sup>21</sup> Therefore, the interaction between viruses and TLRs is critical to the understanding of all viral pathogenesis and immunity.

## TLR Family

It has been surmise most mammalian species have between ten and fifteen type of TLRs. TLR family can be divided into five subfamilies: the TLR2, TLR3, TLR4, TLR5, and TLR9 subfamilies. The TLR2 subfamily is composed of TLR1, TLR2, TLR6, and TLR10. TLR2 forms heterodimers with TLR1, TLR6<sup>22-27</sup> and probably TLR10,<sup>23</sup> each complex having a different ligand specificity. TLR2 recognize a wide range of PAMPs such as, lipoproteins, lipoteichoic acid (Gram native bacterial), lipoarabinomannan (mycobacteria), glycosylphosphatidylinositol anchor (*T. cruzi*), phenol-soluble modulin (*S. epidermis*), zymosan (fungi), glycolipids *Treponema maltophilum*.<sup>22-26</sup> TLR2 are also capable of recognizing viruses, including measles virus (MV), human cytomegalovirus (HCMV), and hepatitis C virus (HCV).<sup>28,29</sup> TLR3 forms a homodimer and recognizes viral double stranded RNA (dsRNA). dsRNA occurred during the viral replication that induced IFN- $\alpha/\beta$  synthesis leading to the anti-viral effect and immune response.<sup>23-26</sup> TLR4 forms a homodimer and recognizes lipopolysaccharide (LPS) from Gram-negative bacteria. The recognition process is enhanced by LPS-binding protein (LBP), which carries LPS to the CD14 molecule, where it is then presented to the MD-2-TLR4 complex.<sup>22-26</sup> The TLR4 complex also recognizes a few other bacterial PAMPs including LTA. Moreover, the TLR4 complex recognizes viruses such as respiratory syncytial virus (RSV), hepatitis C virus (HCV), and mouse mammary tumor virus (MMTV).<sup>22-26,29,30</sup> TLR5 forms a homodimer as well as heterodimer with TLR4. TLR5 recognizes bacterial flagellin of both Gram-positive and Gram-negative bacteria.<sup>31</sup> TLR5 is expressed in epithelial cells of the airways, intestine, and urogenital tract. Interestingly, expression of TLR5 on intestinal epithelium is polarized such that TLR5 is expressed only on the basolateral side of the cell.<sup>32</sup> TLR7 recognizes SYNTHETIC immunomodulators such as imidazoquinolone compounds that are used against human papillomavirus (HPV) infections.<sup>25,26</sup> TLR9 recognizes synthetic CpG oligonucleotides and unmethylated CpG motifs in bacterial and viral DNA, and initiates a signaling cascade leading to the production of proinflammatory cytokines.<sup>22-26,33</sup>

## TLR signaling

The TLR signaling pathways arise from the cytoplasmic Toll/IL-1 receptor (TIR) domain upon ligand binding. The TIR-containing cytosolic adaptor proteins, such as Myeloid differentiation primary response protein 88 (MyD88), TIR-domain containing adapter protein (TIRAP) and TIR domain-containing adaptor inducing IFN- $\beta$  (TRIF), modulate TLR signaling pathways.<sup>34</sup> TLR signaling consists of a MyD88-dependent pathway that is common to all TLRs except TLR3,<sup>35</sup> and a MyD88-independent pathway that is distinguishing to the TLR3 and TLR4 signaling pathways.

In MyD88 dependent pathway, the adapter MyD88, upon stimulation, recruits IL-1 receptor-associated kinase-4 (IRAK-4) to the IL-1 receptor complex. IRAK are active kinases dissociating from the

receptor-adaptor complex upon phosphorylation and associating with TNF receptor-associated factor 6 (TRAF6).<sup>36</sup> TRAF6 then activates at least two distinct signaling pathways leading to the activation of mitogen activated protein kinases (MAPKs) and NF-κB which finally leads to the production of proinflammatory cytokines such as IL-6, TNF and IL-12.

MyD88-independent pathway is dependent on the TIR domain-containing adaptor inducing interferon-β (TRIF)/TIR domain-containing adaptor molecule (TRAM).<sup>37</sup> Receptor-interactor proteins (e.g. RIP1 and RIP3) have been identified as important factor in the pathway which upon stimulation, lead to activation of the transcription factor IRF-3, therefore producing IFN-β. That IFN-β activates Stat1 leading to the induction of several IFN-inducible genes (Figure 1).

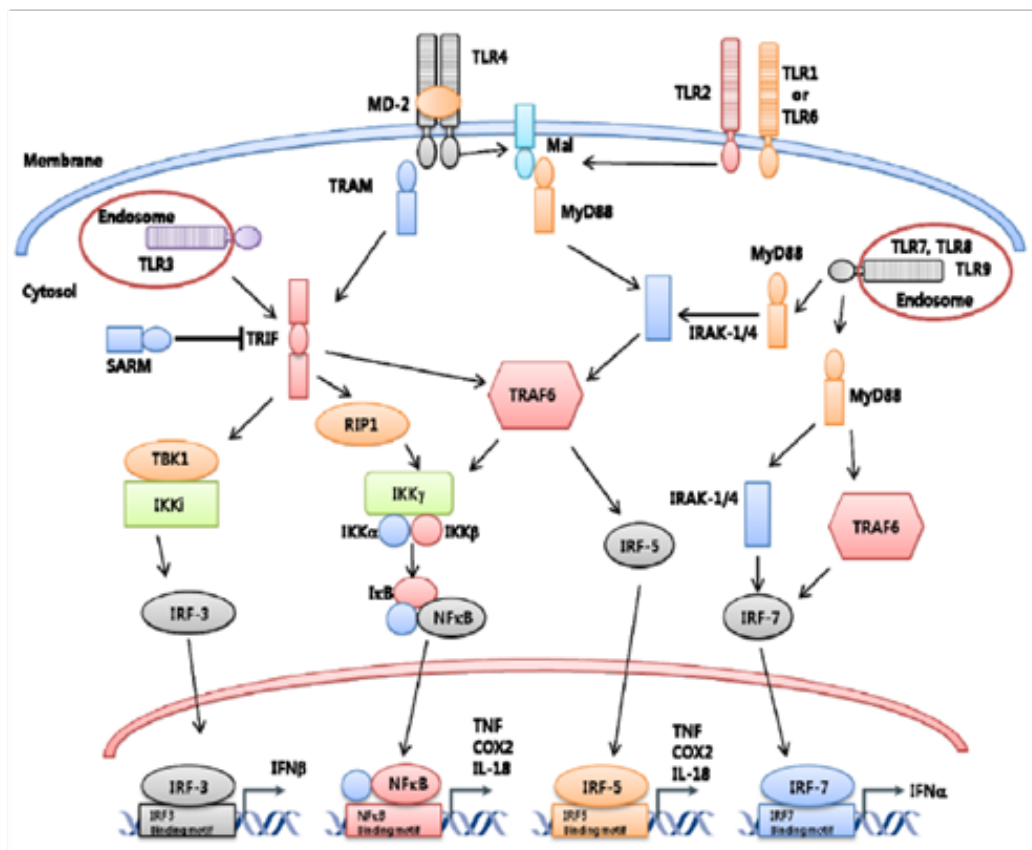


Figure 1 Toll-like receptor signaling.

### Recognition of viruses by TLRs

The specificity of the TLRs for ligands has been studied using bacterial and viral components as well as synthetic ligands.<sup>38</sup> Bacteria are predominantly extracellular pathogens, and therefore, activate host cells predominantly through TLRs expressed on cell surface. However, viruses with their intracellular lifecycle are capable of stimulating the host cells through both intracellular and extracellular

TLRs. The viral components which are usually recognized by different TLRs include dsRNA, ssRNA and CpG-DNA.<sup>39</sup> It is important to note that one type of virus would not be more likely than another to interact with a given TLR. Due to the complexity of viral proteins, it is likely that many viruses will be found to interact with many different TLRs. Based on available literature, a known virus-TLR associations have been shown in (Table 1), however, not all TLRs have been proven, as yet, to interact with viral proteins.

Table 1 TLRs in viral infections of TLR-association anti-viral immunity

Virus genome	Virus	Toll like receptor (or RNA helicases)
Single stranded DNA (ssDNA)	Parvovirus (Adeno-associated )	TLR9
	Herpes Simplex virus	TLR2,TLR3,TLR7,TLR9
	Varicella Zoster Virus	TLR2
	Cytomegalo virus	TLR2/CD14,TLR3,TLR9
Double stranded DNA (dsDNA)	Epstein Barr Virus	TLR2,TLR7
	Vaccinia Virus	TLR2
	Adeno virus	TLR9
	Papillonavirus	MyD88,TLR7

Table Continued....

Virus genome	Virus	Toll like receptor (or RNA helicases)
Single stranded RNA (ssRNA)	Rhinovirus	TLR3
	Encephalomyocarditis virus	MDA5
	Hepatitis A virus	MDA5
	Hepatitis C virus	TLR2, TLR3, TLR4, TLR7
	West Nile virus	TLR3
	Japanese encephalitis	RIG-I
	Dengue virus	RIG-I, MDA5
	Influenza virus	TLR3, TLR7, RIG-I
	Measles virus	TLR2, TLR4
	Respiratory syncytial virus	TLR3, TLR4, TLR7/MyD88, RIG-I
Double stranded RNA (dsRNA)	Sendai virus	TLR7/8, RIG-I
	Reovirus	TLR3, RIG-I, MDA5

## CD4 T helper cell subsets: differentiation and role in viral diseases

### Lineage decisions of CD4 T helper cells

Naive CD4<sup>+</sup> helper T (Th) cells, upon encountering with their cognate antigens presented by professional antigen-presenting cells (APCs), differentiate into effector cells that are characterized by their cytokine production profiles and immune-regulatory functions. The heterogeneity of effector T cells was discovered two decades ago which were then named as Th1 or Th2 cells.<sup>40</sup> Th1 differentiation requires IL-12 and the transcription factors STAT4, STAT1 and T-bet,<sup>41</sup> whereas Th2 differentiation requires IL-4 and the transcription factors STAT6 and GATA3.<sup>42</sup> In addition to Th1 and Th2 cells, a third subset of effector Th cells, Th17, has been identified, the differentiation of which is induced by the combination of TGF- $\beta$  and IL-6.<sup>42</sup> Recently, IL-21 was reported as an autocrine factor induced by IL-6 to regulate Th17 cell differentiation.<sup>43</sup> STAT3, downstream of IL-6 and IL-21, is essential for ROR $\gamma$ t and ROR $\alpha$  expression and Th17 cell differentiation.<sup>42</sup> Additional T cell subsets were also discovered and studied, including T follicular helper (Tfh) cells and IL-9-expressing “Th9” cells, etc. In addition to these effector subsets of CD4<sup>+</sup> helper T (Th) cells, there have distinct regulatory subsets, which in turn suppress adaptive T-cell responses and maintain immune homeostasis. Thymus-derived natural regulatory T (nTreg) cells represent a unique subpopulation of CD4<sup>+</sup> T cells and expression of Foxp3 transcription factor is the hallmark of nTregs, which is required for maintaining Treg cell function.<sup>44</sup> TGF- $\beta$  has been shown to maintain peripheral nTreg cells. Additionally, in the presence of TGF- $\beta$ , Foxp3 can also be induced in naive T cells in the periphery, and the resulting inducible Treg (iTreg) cells exhibit a suppressive phenotype similar to that in nTreg cells.<sup>44</sup> The lineage decisions of CD4 T helper cells are instructed by distinct environmental cytokines which signals through STAT or other inducible but generally ubiquitous transcription factors. Though extensive cross-regulations among lineage-determining transcription factors exist, growing evidence suggests that Th cell lineage commitment can be plastic in certain circumstances.

### The role of CD4 T helper cell subsets in viral encephalitis with special emphasis on Treg and Th17 cells

The presence of the blood-brain barrier in the central nervous

system that restricts entry of cells and protein, the restricted expression of MHC antigens and the nonrenewable nature of the neuronal cell population offer challenges to the immune system for viral clearance and increase the chances for viral persistence. However, vigorous immune responses are mounted rapidly within the CNS against both self and exogenous antigens in the face of antigenic challenge. The lymphocyte subsets which constitute the protective T-cell response within the CNS have not been fully characterized. During viral infection, most CD4<sup>+</sup> T cells isolated from the virus target organ belong to the Th1 type<sup>45</sup> and Th1 cytokines, such as IFN- $\gamma$ , display strong antiviral function and antagonize the development of Th17 cells.<sup>4</sup> In opposition to this protective strategy, a virus may be able to evade antiviral type I and II IFN responses,<sup>46</sup> which facilitate its persistence in the host body by inducing elevated levels of IL-17-producing CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells. This is particularly true in case of chronic viral infection, for example Theiler's murine encephalitis virus infection.<sup>9</sup> However, Th1 cells may also play pathogenic role in viral encephalitis, rather than their protective function which depend on cytokine environment, for example, in case of experimental autoimmune encephalitis, myelin-reactive Th1 preparations devoid of contaminating IL-17<sup>+</sup> cells are highly pathogenic.<sup>5</sup> They can access the non-inflamed CNS, establish the EAE and facilitate the entry of Th17 cells to the CNS during EAE. Th2 cells, the differentiation of which is driven by IL-4, produce the cytokines IL-4, IL-5 and IL-13 and are known to mediate humoral immunity against extracellular pathogens. Humoral immunity driven by B cells with the help of Th2 cells lead to inhibition of virus replication and virus spread in the initial stages. For example, virus neutralizing antibody mediated inhibition of JEV replication leads to the inhibition of the cytopathic effects of the virus and hence less tissue damage in Japanese encephalitis.<sup>47</sup> IL-17-producing Th17 cells, which are a distinct subset of CD4<sup>+</sup> T cells, are apparently involved in inflammatory tissue damage, leading to the pathogenesis of various autoimmune diseases.<sup>4,48</sup> Before the discovery of Th17 lineage of CD4 T cells, Th1 cells were thought to be implicated in the development of autoimmune diseases (AD). However, recent evidence suggests that Th17 cells rather than Th1 cells are the true culprits in the induction and progression of many autoimmune diseases. Furthermore, Th17 cells also appear to play a role in protection against extracellular bacterial or fungal diseases.<sup>11</sup> Th17 cells secrete IL-17A (IL-17), IL-17F, IL-22, IL-6, and TNF- $\alpha$ .<sup>49</sup> IL-17 is a pleiotropic cytokine, which mediates tissue inflammation by inducing many pro-inflammatory cytokines and chemokines.<sup>50,51</sup>

Th17 cells are recently considered as important target cells for the treatment of autoimmune diseases, as they are the primary effector cell type involved in both autoimmune diseases in human and mouse models of these diseases.<sup>52-55</sup> Indeed, expression of IL-17 has been detected in the serum and target tissues of patients with various autoimmune diseases such as RA, multiple sclerosis (MS), and systemic lupus erythematosus (SLE). The production of IL-17 has also been reported in several viral infections, for example, HIV infection in human<sup>6</sup> and herpes simplex virus<sup>7</sup> and respiratory syncytial virus infections<sup>8</sup> in rodents. However, persistent viral infection (eg. Theiler's murine encephalomyelitis virus), preferentially induces the development of Th17 cells, and in turn, these cells uniquely promote viral persistence via IL-17 by inhibiting apoptosis of infected cells as well as by desensitizing target cell killing by T effector cells.<sup>9</sup> Treg, a subset of CD4<sup>+</sup> T cells, are now well recognized for their modulatory function and play pivotal roles in maintaining immune homeostasis and preventing autoimmune diseases.<sup>56,57</sup> The outcome of viral infections is dependent upon complex interactions between the pathogen and host pro- and anti-inflammatory immune responses. Excessive T cell responses have been implicated in disease in mice infected with lymphocytic choriomeningitis virus (LCMV), herpes simplex virus or respiratory syncytial virus, with tissue damage occurring during the process of virus clearance.<sup>58-60</sup> To minimize excessive pro-inflammatory responses, cells with anti-inflammatory activity, such as regulatory T cells and Tr1 cells, which express IL-10,<sup>61</sup> are also induced during a viral infection. An appropriate anti-inflammatory response will prevent immunopathological disease without adversely affecting virus clearance. In healthy individuals, lymphocyte traffic into the CNS is very low and tightly controlled by the highly specialized BBB. However, several pathological conditions of the CNS, such as viral or bacterial infections or inflammation-mediated disorders, induce circulating lymphocytes to cross the BBB and gain access to inflammatory foci.<sup>62</sup> This may be a critical step for Treg to perform their biological function in inflammatory diseases of the CNS. Alternatively, in the absence of Treg extravasation to disease sites, their function could be achieved by soluble factors. Recent studies revealed that several viruses belonging to the Flaviviridae family, such as classical swine fever virus (CSFV), Dengue virus (DV), Japanese encephalitis virus (JEV) and Yellow fever virus (YFV), infected DCs and altered the cell phenotype and function.<sup>63-65</sup> Furthermore, Aleyas et al.,<sup>66</sup> recently reported that JEV Beijing-strain replicated both in bmDCs and macrophages, and induced functional impairment of DCs through MyD88-dependent and independent pathways which subsequently led to poor CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses.<sup>66</sup> These findings suggest that the virus-induced alteration of DCs is a likely cause of the immunosuppression mediated by Tregs. The neuronal protection by Tregs was found to be mediated in several viral-encephalitis such as human HIV-1 encephalitis<sup>67</sup> and Japanese encephalitis.<sup>10</sup> In Japanese encephalitis, the infection of DCs with JEV P3 expanded the population of CD4<sup>+</sup> Foxp3<sup>+</sup> regulatory T cell (Treg) with immunosuppressive potential, suggesting that the virus-induced alteration of DCs in JEV infection is likely cause of the immunosuppression by iTregs.<sup>10</sup> However, the detailed immune evasion mechanism in viral encephalitis is still not fully circumvented.

### Plasticity of Th17 and Treg cells and their balance in viral encephalitis

A balance between Th17 and Treg is crucial for immune homeostasis and the plasticity in the Th17 and Treg developmental programs play pivotal role in the development of balanced immune response through regulated differentiation of anti-inflammatory Tregs and proinflammatory Th17 cells from naïve precursors. Though,

natural Tregs (nTregs) develop during thymic selection through a mechanism independent of TGF- $\beta$ , the extrathymic development of induced Tregs (iTregs), is TGF- $\beta$ -dependent. Development of Th17 cells is also TGF- $\beta$ -dependent, but additional coordinate signaling by IL-6, produced by dendritic cells activated by microbial products, is required in concert with TGF- $\beta$  to induce Th17 differentiation. Thus, in the absence of proinflammatory signals from the innate immune system, priming of naïve CD4 T cells by antigen in the presence of active TGF- $\beta$  promotes development of iTregs, whereas activation in an environment where both active TGF- $\beta$  and IL-6 are available promotes Th17 development. Because TGF- $\beta$  suppresses Th1 and Th2 differentiation,<sup>68</sup> iTreg and Th17 development are favored in its presence. The transcription factor central to Th17 differentiation is an isoform of the retinoic acid-related orphan receptor  $\gamma$ , ROR $\gamma$ , which is expressed in T cells (ROR $\gamma$ t)<sup>69</sup> and directs the differentiation program of proinflammatory IL-17<sup>+</sup> T helper cells. A second member of the ROR family, ROR $\alpha$ , is also associated with the Th17 development program<sup>70</sup> but its contribution appears dispensable. On the other hand, the transcription factor central to Treg differentiation and function is Foxp3. Interestingly, naïve T cells stimulated with TGF- $\beta$  alone were found to upregulate both Foxp3 and ROR $\gamma$ t, however, as Foxp3 is able to associate with ROR $\gamma$ t and inhibit ROR $\gamma$ t transcriptional activation, treatment of naïve T cells with TGF- $\beta$  led exclusively to Treg differentiation.<sup>71</sup> Therefore, the balance of TGF- $\beta$  and IL-6 signaling might determine the differentiation of iTregs or Th17 cells through antagonistic competition of Foxp3 and ROR $\gamma$ t (Figure 2). Several studies recently reported that all-trans retinoic acid (at-RA), derived from Vitamin A, can potently inhibit Th17 development and promote iTreg development, at least in part by antagonizing the effects of IL-6.<sup>72-74</sup> This implies that interactions between Foxp3 and ROR factors could be directly or indirectly modulated by the binding of retinoic acid to the retinoic acid receptor, RAR, although specific mechanisms by which this might occur are unclear. Thus, IL-6 acts as a potent pro-inflammatory cytokine in T cells through promotion of Th17 differentiation and inhibiting Treg differentiation, indicating that the control of IL-6 may normalize the balance between Th17 and Treg in viral encephalitis and may alleviate clinical severity.

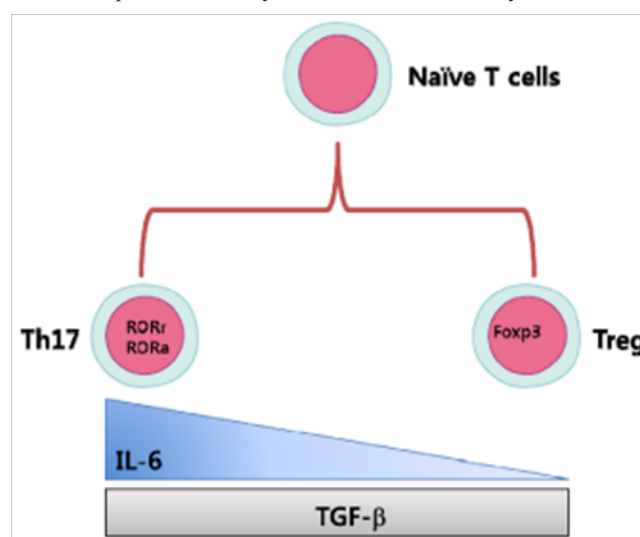


Figure 2 IL-6 mediates Th17/Treg balance.

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## Conflict of interest

The author declares no conflict of interest.

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