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Japanese Encephalitis Virus: Innate and Adaptive Immunity

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1. Introduction

Japanese encephalitis virus (JEV) is a flavivirus that is one of the major causes of encephalitis in Eastern and Southern Asia with recent observations marking its spread to newer geographical regions. Flaviviruses, in general consitutute a global health threat since many of them are endemic in large parts of the Americas, Africa and Australia in addition to Asia and suitable vaccines are unavailable for many of them. JEV causes serious inflammation of the brain, which may lead to permanent brain damage, and has a high mortality rate. More than three billion people live in JE endemic areas and JEV is estimated to cause 45,000 human cases of disease and 10000 deaths per year (Solomon and Winter, 2004; van den Hurk et al., 2009).

The occurrence of encephalitic epidemics in humans due to this virus have been recognized from 1870s in Japan where it was termed Japanese B encephalitis to distinguish it from type A encephalitis caused by sleeping sickness. The first prototype Nakayama strain was isolated and established in 1935 from the brain of a fatal case from Tokyo, Japan. This was closely followed by virus isolation from *Culex tritaeniorrhynchus* in 1936 confirming earlier suspicions that mosquitoes served as the transmission vector (Solomon et al., 2000).

JEV transmission occurs through a zoonotic cycle involving mosquitoes as an important intermediate maintenance and replicative vector. Vertebrates, chiefly pigs and ardeid birds act as amplifying hosts. Only those animals that develop high viraemias are significant in this natural enzootic cycle. Humans are infected only coincidently when bitten by an infected mosquito and are dead end hosts. Human to human transmission is yet to be documented, possibly due to the presence of transient viraemia. The absence of high serum viraemia prevents the virus from being picked up during mosquito bites. Pigs play a major role in the transmission cycle with respect to human infection since they are often bred close to humans whereas herons, egrets and other ardeid birds are important for maintenance and overwintering of this virus. Among the other vertebrates, horses also develop central nervous system (CNS) infections and are considered as dead end hosts. Rodents are relatively refractory to infections while amphibians, reptiles and bats can also be infected (Mackenzie et al., 2004). JEV does not cause encephalitis in pigs and birds although it is

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known to result in abortions in pigs (D.S. Burke and Leake, 1988). Thus the incidence of JEV induced disease correlates with rising mosquito densities and increased human contact with amplifying vertebrate hosts. As a result, activities such as deforestation and irrigation that are associated with farming as well as increases in temperatures and precipitation that occurs during monsoons favor JEV infection and propagation. Not surprisingly, seasonal JEV epidemics are associated in the tropics with the beginning of the monsoon season (Umenai et al., 1985). The increased rainfall during the monsoon season has been shown to be followed by an increase in mosquito breeding leading to seroconversion in farm animals and later human encephalitis (Mani et al., 1991) in Southern India as well as in other northern areas of Vietnam, Thailand and Korea, Japan and China (Erlanger et al., 2009; Hoke et al., 1988).
Under laboratory conditions *Culex* species *Cx. tritaeniorrhynchus, Cx. geldius, Cx. pseudovishnui and Cx. fuscocephala* have found to be very efficient vectors for JEV transmission (Solomon, 2006; Solomon, 2004). However, JEV has also been isolated from other species of mosquitoes as well. JEV has been isolated from ten different species of *Culex*, four different species of *Anopheles* and three different species of *Mansonia* mosquitoes (Reuben and Gajanana, 1977). Among various *Culex* species, *Cx. tritaeniorrhychus* has been found to be a very efficient vector for JEV replication and transmission. It breeds predominantly in paddy fields and is the primary vector for transmission of JEV throughout Southeast Asia (Takahashi, 1976).

2. Epidemiology

A major epidemic involving encephalitis was observed in Japan in 1924 with over 6000 cases and 3797 deaths which was followed by another large outbreak in 1935 and then, annual outbreaks from 1946 to 1952. Subsequently, outbreaks were reported in China in 1940 and in Korea in 1949 and in other Asian countries which include Thailand, Vietnam, Burma, Bangladesh, Indonesia, Malaysia, the Philippines, Sri Lanka, India, and Taiwan where epidemics have been reported since 1950s. JE was reported from Russia in 1938 while a large epidemic was reported in North Vietnam and North Thailand in the 1960s (Endy and Nisalak, 2002).
JE is now being described from newer geographical areas and was reported from Australian Torres straits island as well as the mainland in 1995 and 1998 (Mackenzie et al., 2002; Hanna et al., 1999). The factors contributing to this geographical spread could be multifold including several environmental and ecological factors as well as the ability of humans to travel and birds to migrate. The former was exemplified by the isolation of another flavivirus, WNV from New York in 1999 (Lanciotti et al., 1999).
In India, JEV was first diagnosed in 1955 in Vellore, North Arcot district of Tamil Nadu (Kabilan et al., 2004). Major outbreaks were reported in West Bengal in 1973 with 700 cases and 300 deaths. This was followed by another epidemic in 1976 in the same region. Other outbreaks have been reported in several other states of India which include Bihar, Andhra Pradesh, Assam, Manipur, Uttar Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Haryana (Kabilan et al., 2004).
Broadly three epidemiological regions have been associated with JE (Rodhain, 1996):

1. Temperate epidemic region: In temperate regions such as Northern China, Korea, Japan, Taiwan and Southern Russia, the extreme low temperatures of the winters do not allow mosquito transmission. However, in the summer and autumn months, the thaw and increase in temperatures allows mosquito transmission, possibly leading to large epidemics.

2. Endemic region: In southern regions of India, Southern Vietnam, Thailand, the Phillipines, Malayasia and Indonesia, JEV is endemic due to the constant presence of mosquitoes and the bird-mosquito-pig cycle gains predominance.
3. Intermediate subtropical region: In Northern India, Burma, Northern Thailand, Northern Vietnam and Southern China and Bangladesh, JEV transmission occurs continuously at low levels. However, it increases soon after the monsoon and rainy season. Unexposed children are mostly affected in such regions since the adults in the region would have been exposed at some time to the virus.

JEV causes disease mostly in new born children and young adults. Most infections in adults are asymptomatic, at most having flu-like symptoms and rarely cause disease. However, immunologically naïve adults are susceptible to the disease and they could be affected when epidemics occur for the first time in their geographical area. The estimated ratio of symptomatic to asymptomatic infection varies from 1 in 25 to 1 in 1000 (Solomon and Winter, 2004). The incidence of symptomatic JE infection is influenced by several factors including previous exposure to flaviviruses, age-related differences, different immunological and genetic factors as well as efficiency of available surveillance procedures. The occurrence of high levels of neutralizing antibodies in children aged above 14 years, possibly due to natural exposure and subclinical infections decreases the susceptibility of these older age groups.

3. Clinical features

JEV normally infects children below 15 years and man to man transmission of JEV is not detected. The incubation period after the infected mosquito bite is not exactly known, but varies from 1-6 days normally. The onset of illness can be abrupt, acute, subacute or gradual. Progression of disease can be divided into three stages: (i) the prodromal stage preceding CNS features (ii) an encephalitis stage marked by CNS symptoms and (iii) the late stage noticeable by recovery or persistence of signs of CNS injury (Misra and Kalita, 2010; Huy et al., 1994; Kumar et al., 1993). Definitive clinical diagnosis is not possible in prodromal stage, but is characterized by high grade fever, with or without rigors, headache, general malaise, nausea and vomiting. The prodromal stage usually lasts 1-6 days. It can be as short as less than 24 hr or as long as 14 days. The initial symptoms in children are usually nausea, vomiting and abdominal pains that are associated with acute abdominal syndromes. The encephalitic stage is characterized by altered sensorium, convulsions, neck stiffness, muscular rigidity and abnormal movements, speech impairment, mask-like face, tremors in finger, eyes, eyelids and tongue. The late stage is characterized by the persistent signs of CNS injury such as mental impairment, increased deep tendon reflexes, paresis either of the upper or lower motor neuron type, epilepsy, abnormal movements and behavioral abnormalities. Case fatality rates for JEV range from 0-30% (Burke et al., 1985). Children who survive take several weeks to regain the neurological functions. However, only one third of the affected children recover the normal neurological functions. 30% of survivors have persistent motor deficits like weakness of upper and lower motor neurons. 20% of the patients suffer from severe cognitive and language impairment and some patients show further convulsions. Some patients are observed to exhibit mild sequelae such as learning disabilities and behavioral problems (Kumar et al., 1993). Chronic progressive encephalitis and relapse, caused by persistence of virus in the CNS has been reported in a small number of patients (Pradhan et al., 2001; Ravi et al., 1993). The details involving the clinical presentation of JE have been reviewed (Misra and Kalita, 2010).

4. Structural and genome organization

4.1 RNA genome

JEV is a RNA virus belonging to the *Flavivirus* genus of the family *Flaviviridae.* The *Flaviviridae* is a large family of viruses responsible for causing severe disease and mortality in humans and animals. The family consists of three genera: *Flavivirus, Pestivirus* and *Hepacivirus.* Among the three, *Flavivirus* is the largest genus and includes several viruses including dengue virus (DNV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), West Nile virus (WNV) and yellow fever virus (YFV). Most members of the *Flavivirus* genus are arthropod-borne or arboviruses, which indicates the requirement of a blood sucking arthropod to complete their life cycle. Few of the members have no known vector. The genus contains over 70 viruses, of which approximately 40 are mosquito borne, 16 are tick borne and 18 have no known vector (Heinz et al., 2000). Flaviviruses are serologically related and are classified based on cross-neutralization assays using polyclonal sera into 12 sero-complexes (D.S Burke and Monath, 2001; Gubler, 2002). The important ones are the dengue serological group, Japanese encephalitis serological group and yellow fever virus group. JEV belongs to the JE serocomplex which also includes St. Louis encephalitis virus (SLE), West Nile Virus (WNV), Kunjin virus (KUNV), Murray Valley encephalitis virus (MVEV), Cacipacore virus (CPCV), Kautango virus (LOUV), Alfuy virus (ALFV), Usutu virus (USUV) and Yaounde virus (YAOV). JEV has been grouped into 5 genotypes (I -V) based on the phylogenetic analysis of PrM and E encoding genes and different genotypes are found in different climatic regions (Heinz et al., 2000; Nitatpattana et al., 2008; Solomon, 2006).

The complete nucleotide sequence of JEV was reported in 1990 (McMinn, 1997) and shows that it consists of a single stranded, positive-polarity 11kb long RNA. The 3′ end of the genome lacks a poly-A-tail and a type 1 cap (m7GpppAmp) is found at the 5′ end. Flaviviral genome RNAs are also characterized by their high purine content and low CG and UA doublet frequencies (Rice et al., 1985). Untranslated regions (UTR) that contain highly conserved sequences and secondary structures are located at the 5′ and 3′ end of the JEV genome and are 95 and 585 nucleotides long respectively (Sumiyoshi et al., 1987). Host cell and viral proteins are involved in the conversion of the positive sense RNA into a negative sense RNA through a double stranded replicative form (dsRF) after the first round of translation. The negative sense RNA acts as a template for the synthesis of progeny positive sense RNAs that is packaged with viral proteins and assembled into infectious virus particles. Approximately 10-100 fold excess plus strands exist relative to negative strands at any given time within an infected cell. Several cellular and viral proteins such as EF-1α (Davis et al., 2007), TIA-1 (Li et al., 2002) and YB-1 (Paranjape and Harris, 2007) have been reported to bind the UTRs and regulate flaviviral translation and replication. In the case of JEV, FBP1 represses JEV protein expression by interacting with the 3′ and 5′ UTRs (Chien et al., 2011) and La autoantigen facilitates replication by interacting with the 3′ UTR. The role of various host cell factors in flaviviral infection and replication has been reviewed in detail elsewhere (Pastorino et al., 2010).

The RNA genome of JEV contains a single long open reading frame that codes for a polyprotein which is cleaved by host and viral encoded proteases to form three structural and seven non-structural proteins (Fig 1). The N-terminal end of the polyprotein encodes the three structural proteins called capsid (C), a glycosylated membrane protein precursor (prM) and an envelope protein (E). The prM is cleaved to precursor (pr) and membrane protein (M) during JEV maturation. These structural proteins are followed by seven non-structural (NS) proteins (NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5) (Chambers et al., 1990). The structural proteins finally become components of the mature infectious viral particle

while the non-structural proteins take part in the process of polyprotein processing, viral RNA synthesis and virus morphogenesis within the infected cell. Signal sequences direct the polyprotein precursor into the host endoplasmic reticulum (ER) with NS1 and the exogenous domains of prM and E facing the lumen while C, NS3 and NS5 are cytoplasmic. The mature viral proteins are formed by post and cotranslational cleavage of the polyprotein by NS3 in the cytoplasm and ER resident host proteases. Both the processes of polyprotein processing and RNA replication are thought to occur in distinct but adjacent compartments composed of convoluted membranes and vesicle packets containing dsRNA (Salonen et al., 2005; Welsch et al., 2009). The process of virus assembly occurs in the lumen of the rough endoplasmic reticulum (RER) while the final maturation of virus particles occurs in the trans-golgi network and involves the cleavage of prM to M by furin-like protease. This is a step that causes conformational rearrangements of the E protein and is essential for converting fusion incompetent, non-infectious virus particles to mature infectious virions (Yu et al., 2008). These mature viruses exit the host cell as packages of virions.

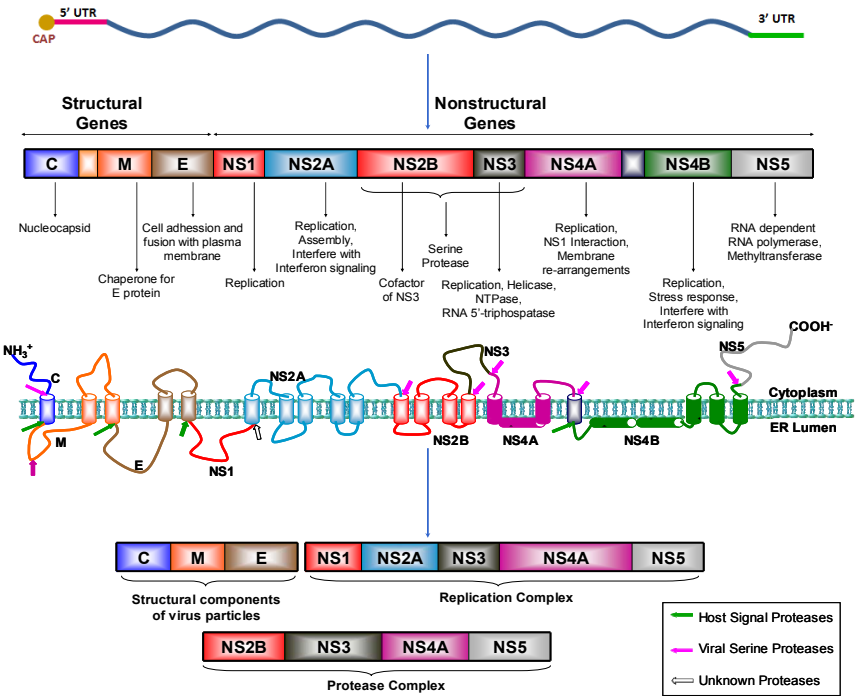

Fig. 1. Schematic diagram of Japanese encephalitis virus genome and polyprotein organization. 11 kb ssRNA JEV genome can be divided into four regions – 5′Cap and 5′UTR, Structural Protein coding gene regions, Nonstructural Protein coding gene regions and 3′UTR. It codes for ~ 380 kDa polyprotein which is further processed by several host and viral proteases to give rise to individual proteins. These individual proteins can assemble together to give rise to structural complexes made up of capsid (C), matrix (M) and envelope (E) proteins. The nonstructural proteins take part in virus replication and assembly within infected cells but do not form part of the mature virus particle that is released (adapted from Assenberg et al., J. Virol (2009) 83: 12895-12906

The RNA genome is packed into a particle by the viral capsid protein (C) in a host-derived lipid bilayer containing the viral envelope protein (E) that participates in receptor binding, membrane fusion and viral assembly. The virus gains access to local fibroblasts when it is deposited at a local site during the mosquito bite where it replicates leading to transient serum viraemia. Both hsp70 (Das et al., 2009b) and integrin molecules (Chu and Ng, 2004) have been shown to act as viral receptors for JEV and WNV respectively. Subsequent viral entry occurs by receptor mediated endocytosis (Lee et al., 2008) involving clathrin coated vesicles (Nawa et al., 2003). Low endosomal pH induces a structural reorganization of the E protein and mediates the fusion of the viral and host cell membranes resulting in the release of the viral nucleocapsid into the cytoplasm (Rey et al., 1995). Infectious virus is released 12 hrs post infection (p.i.) as studied in infected cell lines but viral RNA synthesis can be observed as early as 3 hrs after infection and host macromolecular synthesis is not significantly inhibited by the virus at these times. Although a wide variety of host cells including arthropod cells support virus infection, monocytes, macrophages and dendritic cells are believed to be the main cells that support the spread of this virus into the visceral organs and thence into the CNS in vertebrates. Maximum virus titres vary considerably from cell type to cell type. Although the virus infections in arthropod cells are generally noncytopathic, they are cytocidal in vertebrate cells (Chambers et al., 1990). The period of transient viraemia is followed by the viral invasion of the CNS either through hematogenous spread or by the breakage of the blood-brain barrier and entry through endothelial cells in the cerebrum (Liu et al., 2008). CNS infection is associated with perivascular cuffing that is rich in B cells and the infected parenchyma is invaded by inflammatory macrophages that enter to phagocytose infected and dying cells. Parenchymal damage associated with JEV infection is mainly due to the inflammatory reactions triggered by such invading leukocytes as well as the direct virus- induced cellular apoptotic processes that occur within the CNS.

4.2 Viral proteins

4.2.1 The Capsid and matrix protein

The C protein is highly basic in nature and forms the nucleocapsid along with the genomic RNA. The C protein is a small (12-14 kDa) highly positively charged protein that contains a C-terminal hydrophobic region that is preceded by a hydrophilic region and a central hydrophobic region. It is known to have both N-terminal and C-terminal modifications (Chambers et al., 1990). Cathepsin L mediated processing of this protein has been shown to play an important role in JEV replication in neural and macrophage cells (Mori et al., 2007). Monoclonal antibodies that have been made to the Dengue 4 virus C protein have been shown to localize in the nucleus of the infected cell (Tadano et al., 1989) and nuclear localization of C has been shown to enhance JEV replication in infected cells (Mori et al., 2005). The prM (~26 kDa) is a glycoprotein precursor to the M structural protein and possesses several N-linked glycosylation sites that are conserved in the JE serogroup while the N terminal 'pr' segment contains six conserved cysteine residues, all of which form disulfide bridges (Chambers et al., 1990). A single N-linked glycosylation site has been identified on prM that is essential for the release of JE virus particles from infected cells as well as its ability to cause pathogenesis (Kim et al., 2008). A valine to alanine change at aa76 has been associated with escape from antibody neutralization in MVE (McMinn et al., 1995). Anti-prM antibodies were shown to have low virus-neutralizing activity (Takegami et al., 1982) but its biological significance has not been elucidated. The role of C protein in eliciting B cell responses is yet unclear.

4.2.2 The envelope protein

The envelope (E) protein (~ 53 kDa) is the major virion surface protein with 180 copies existing as head to tail dimers on the virus surface as shown for Dengue viruses (Kuhn et al., 2002). It contains about 500 amino acids and two potential glycosylation sites and mediates viral attachment and fusion to cell membranes of receptor-bearing cells. These properties of E protein are responsible for its glycosylation as well as generating virus neutralizing, haemagglutination inhibiting and fusion blocking antibodies. A feature that distinguishes this structure from that of many others is that the orientation of the E-head to tail homodimers is parallel rather than perpendicular to the membrane as would be expected of a viral glycoprotein spike (Heinz and Allison, 2000). The formation of the E protein homodimer found in the mature virus particle takes place just before the release of the mature virus particle and is associated with the cleavage of the closely associated prM protein to M by a furin-like protease (Yu et al., 2008). While the immunogenicity of the flaviviral E protein has led to the generation of many monoclonal antibodies that were used to understand virus structure, they have also been used to evaluate antigenic drift of related viruses, analysis of the replication cycle as well as in virus detection and diagnostic tools (Roehrig, 2003).

The crystal structure of E protein of JEV has been deduced from knowledge based homology modeling approach using 2Å crystal structure of the E protein of TBEV (Rey et al., 1995) as template (Kolaskar and Kulkarni-Kale, 1999) as well as recombinant subviral particles (RSPs). The RSPs were found to be icosahedral with 30 dimers arranged in a T=1 lattice. Lateral dimer-dimer interactions appear to involve contacts between a loop in domain II on one side and a groove in domains I and III on the other side. The crystal structure of E protein of the Dengue virus type 2 S1 strain has also been found to be nearly identical to that of TBEV protein (Modis et al., 2003). In addition, all of the 12 cysteines present in TBEV and the six disulfide bonds assigned to them are absolutely conserved. Three distinct domains (I, II, III) were elucidated in the E protein and this structure has been assumed for all flavivirus E proteins. These domains directly correspond to the antigenic domains C, A and B respectively, which had been previously defined using monoclonal antibodies to examine the antigenic structure of the TBEV envelope protein. Based on the E protein model of Kolaskar and Kulkarni (1999), domain I contains 128 amino acid residues from 1-51,137-196 and 293-311. The second domain (II) consists of 171 amino acid residues from 52-136 and 197-292 and the IIIrd domain is a contiguous stretch of 100 amino acid residues from 310-411. Domain III contains an IgC immunoglobulin-like domain (Lin and Wu, 2003), that is a unique feature not found in other viral envelopes and an integrin binding motif RGD at aa387-389. This integrin binding motif has been suggested to be involved in viral attachment to the target cell (Mandl et al., 2000; Rey et al., 1995) while amino acids 98-110 of domain II has been suggested to form a barrel shaped fusion peptide that is responsible for initiating the fusion of the virus to endosomal target membranes (Allison et al., 2001; Rey et al., 1995). A unique transmembrane hairpin present in the E protein is needed for this membrane fusion event (Fritz et al., 2011). Recently, a broadly flavivirus cross-neutralizing epitope has been characterized within this fusion loop (Deng et al., 2011). Thus, many of the JEV neutralizing epitopes/regions have been found in these two domains with predominance in Domain III (Table 1). Peptides from this region have also been useful for serological diagnosis (Chavez et al., 2010). However, the binding site of a strongly neutralizing monoclonal antibody (mAb) against JEV (Kimura-Kuroda and Yasui, 1983) was shown to be at the junction area of the Domain I and II and being distant from the virus-target cell attachment site (Morita et al., 2001). Several neutralizing antibodies have

been shown to bind the C-terminal E region aa280-414 (Mason et al., 1987). Fragment (aa205-456) that was obtained by CNBr cleavage of the purified E protein has also been shown to induce neutralizing antibodies (Srivastava et al., 1990). Overall, six neutralization regions have been identified based on escape variant analysis of different flaviviruses including JEV. These are region 1, aa66-72 and 112; region 2, aa123-128; region 3, aa155 and 158; region 4, aa171, 181 and 293, region 5, aa52, 136 and 270 -279; region 6, aa307-311, 333 and 384-385. These regions are found on the outer, upper or lateral surfaces of the E-glycoprotein. Many of these regions of the E protein that are associated with neutralizing epitopes have been used in various immunization approaches to develop vaccines and have been detailed later in the chapter.

Envelope Domain	Amino Acid Number	Property of neutralization epitope/region	Reference
I, III	303-396	Binds several anti-JEV neutralizing antibodies	(Mason et al., 1989)
II, III III	205-456 375-457	Fragments obtained by CNBr cleavage Induces neutralizing antibodies in mice	(Srivastava et al., 1990)
III	319-500	Protein A fusion protein eluted from electrophoretic gels induces neutralizing antibodies and protection in mice	(Srivastava et al., 1991)
II III	270 333	Monoclonal antibody neutralization escape variant	(Cecilia and Gould, 1991)
III III	373-500 373-399	Protein A or GST fusion peptide induces neutralizing antibodies	(Seif, Morita, and Igarashi, 1996; Seif et al., 1995)
II II II II	52 126 136 275	Monoclonal antibody neutralization escape variant	(Morita et al., 2001)
I	149-163	Monoclonal antibody raised to synthetic peptide E149-163 neutralizes JEV	(Dewasthaly et al., 2001)
I II III	307-309 327-333 386-390	Phage-display libraries used to select neutralizing peptide ligands	(Wu et al., 2004)
I, III	292-500	Purified E292-500 induces neutralizing antibodies and protects in mice	(Chia et al., 2001)
III	373-399	E373-399 expressed as a fusion protein with JGM virus like particles induce neutralizing antibodies and protection in mice	(Saini and Vrati, 2003)

Table 1. Some Neutralization Epitopes/Regions in the E protein of JEV

4.2.3 The NS1 protein

NS1 is a secreted glycoprotein that is highly conserved across flaviviruses and has been hypothesized to aid in immune evasion. Flaviviral NS1 has been shown to inhibit complement activation both in solution and on cell surfaces (Chung et al., 2006a) due to its

ability to bind and recruit the complement regulatory protein factor H. The protein is highly immunogenic and some studies report the protective nature of anti-NS1 antibodies both by immunization and passive transfer (Chung et al., 2006b; Schlesinger et al., 1986). The NS1 protein is not incorporated into the mature virion and has been found in the serum of infected individuals and animals (Alcon et al., 2002) but its high immunogenicity, serotype-specific nature of the antibodies and the identification of a conserved B cell epitope on NS1 of JEV suggests that NS1 is a virus-specific serological antigen (Wang et al., 2009). Monoclonal antibodies have been used to define antigenic domains at the N and C- termini of JEV NS1. Antibody-based assays using anti-NS1 antibodies have also been designed to differentiate between JEV and WNV infections (Kitai et al., 2011).

Multiple forms of NS1 are present in flavivirus infected cells and a protein containing NS1 and a portion of NS2A (NS1′) is found within cells and extracellular fluid of JEV infected cells. The membrane associated forms are hypothesized to form during its dimerization and are detected after 30 minutes of viral protein synthesis (Chambers et al., 1990). It has been shown to be a GPI anchored protein (Jacobs et al., 2000; Winkler et al., 1989) and the GPI anchor is believed to be added to the C-terminus by an ER resident enzyme.

The JE serocomplex NS1 contains 12 conserved cysteines that form disulfide bonds and three N-linked glycosylation sites. Two of these sites are conserved in all mosquito-borne flaviviruses and correspondingly, at least two N-linked oligosaccharide chains are present in the cell-associated and extracellular NS1 and NS1′ in JEV infected cells. Mutations in these N-linked glycosylation sites led to significant defects in RNA replication indicating its important role in virus replication (Chambers et al., 1990).

NS1 (48 kDa) induces a strong antibody response due to its presence both on the surface as well as in secretory form, apart from being present intracellularly in virus induced vesicle packets (Fan and Mason, 1990) and extracellular nonvirion forms. This ability of NS1 to induce a strong antibody response prompted its evaluation for protective efficacy in mice models immunized with either recombinant vaccinia virus (VACV) expressing the NS1 gene or recombinant DNA based immunizations (Lin et al., 1998; Konishi et al., 1991). These results indicate that immunizations with NS1 can provide only low protection from JEV infection, as compared to that elicited by the E protein alone. The secreted form has been named as the soluble complement fixing (SCF) antigen due to its ability to fix complement (Brandt et al., 1970). A recent study has shown that the *E. coli* expressed NS1 protein induces protective humoral immune response in mice during JEV infection (Lin et al., 2008b). A synthetic NS1 peptide has been shown to reconstitute lysis of uninfected syngeneic targets by anti-JEV specific CTL (Murali-Krishna et al., 1996) and anti NS1 antibodies have been shown to reduce virus production from infected cells (Krishna et al., 2009).

4.2.4 The NS2A & NS2B protein

The NS2 protein has two relatively hydrophobic components – NS2A (~25 kDa) and NS2B (~16 kDa) with short hydrophilic segments. NS2A forms part of the replication complex, inhibits IFN production (Liu et al., 2006a) and is also involved in the biogenesis of Kunjin virus-induced membranes that take part in virus assembly (Leung et al., 2008). It has also been shown to bind to the 3′ UTR of viral RNA (Mackenzie et al., 1998). Little information is available regarding NS2A from JEV, but studies with YFV and KUNV suggests that this membrane spanning protein is generated when NS1-2A is cleaved by an ER resident protease at the N-terminal end while its C-terminus is generated by virus encoded NS3 serine protease in the cytoplasm (Falgout and Markoff, 1995). Mutants that block the latter

cleavage as well as immunogold localization studies suggest an important role for NS2A in virus replication. Mutations at Lys190 of NS2A and other suppressor mutations in NS3 suggest that NS2A interacts with NS3 in infectious particle production. Similarly, the NS2B protein also complexes with NS3 and is a required cofactor for NS3 serine protease activity as well as its proper folding. NS2B has also been suggested to modulate membrane permeability during JEV infection. Very little information is available regarding their antigenic structure but other studies suggest that NS2A and NS2B may take part in viral RNA assembly (Leung et al., 2008; Lindenbach and Rice, 2003; Kummerer and Rice, 2002).

4.2.5 The NS3 protein

The NS3 protein is the second largest viral protein (70 kDa) and is a multifunctional membrane-associated protein with protease, helicase and RNA triphosphatase activities. The serine protease is required for polyprotein processing (Pastorino et al., 2010; Assenberg et al., 2009) while the helicase/NTPase activity is needed for unwinding the double stranded replicative form of RNA and the RNA triphosphatase activity is needed for capping nascent viral RNA (Bollati et al., 2009). Much of the available information about NS3 has been derived from crystallographic studies of the MVEV protein that is a member of the JEV serogroup. Recent structural data reveal that this single protein has two segregated domains connected by a linker domain (residues 169-181). The relative orientation of these domains differs between different flaviviruses and the helicase and protease activities are independent of each other (Assenberg et al., 2009).

Residues 1 to 169 mediate the trypsin-like serine protease activity and contain the characteristic catalytic triad (Asp-His-Ser) as well as a highly specific substrate recognition sequence that is conserved in all flaviviruses (Chambers et al., 1990). The presence of an aberrant fold makes its structure different from the canonical trypsin structure and makes it dependent on a noncovalent association with a 47 amino acid hydrophilic stretch of NS2B for its proteolytic activity. NS3 cleaves at the C-terminal side of highly conserved dibasic residues located between NS2A/NS2B, NS2B/NS3, NS3/NS4A, NS4B/NS5.

Residues 170 to 619 mediate the activities of RNA strand separation and (poly)nucleotide hydrolysis. The structure of this region has been solved for JEV (Yamashita et al., 2008) and other flaviviruses (Luo et al., 2008; Wu et al., 2005). These studies show that NS3 is a member of the DEAH/D family of helicase superfamily (SF2) containing the Walker A and B motifs that are responsible for NTP and Mg^{2+} binding. It's abilities to separate nascent from template RNA strands and to unwind RNA secondary structure in the 3′ untranslated region is presumed to assist RNA replication. The RNA 5′ triphosphatase activity is thought to facilitate viral RNA capping by removing the terminal γ-phosphate. In addition to its interaction with NS2B which is a cofactor required for its proteolytic activity, NS3 has also been shown to interact with NS5 as a result of which its helicase/NTPase activity is stimulated. The NS3 protein of JEV has also been shown to interact with Src resulting in NS3 tyrosine phosphorylation although the significance of this phosphorylation is still unclear (Raung et al., 2007). Thus, its multifunctional activities have made NS3 an attractive target for antiviral strategies.

4.2.6 The NS4A & NS4B protein

Flaviviruses mediate late cytoplasmic membrane rearrangements that occur after the virus latency period in order to facilitate efficient RNA replication (Westaway et al., 1997). Such rearrangements that involve microtubules and microtubule-associated proteins may

facilitate efficient JEV replication by enabling intracellular trafficking of viral components. A certain threshold of viral RNA replication was found to be required for the formation of these structures indicating that cellular membrane biogenesis was closely linked to viral RNA replication and protein synthesis. Three types of membrane structures are induced called convoluted membranes (CM), paracrystalline arrays (PC) and vesicle packets. The CMs are derived from the RER while the vesicle packets are derived from the trans-golgi region of infected host cells. Antibody staining for virus and host-specific proteins reveals that each of these structures perform specific functions during viral replication. Although not shown for JEV, studies with KUNV and WNV suggest that flaviviral NS4A protein mediates these cytoplasmic membrane rearrangements. The cleavage of the NS4A-4B polyprotein intermediate by the NS3-2B protease serves as the signal for the induction of cellular membrane biogenesis and allows NS4A targeting to the golgi complex where viral replication occurs. The exact nature of NS4A cleavage fragments that are involved in these processes as well as the role of NS4A and NS4B in viral replication is still unclear (Roosendaal et al., 2006). Information about posttranslational modifications despite the presence of a conserved, potential N-linked glycosylation site in the C-terminal portion of NS4B is also not clear (Chambers et al., 1990). However, it has recently been demonstrated that the peptidyl-prolyl isomerase (PPIase) function of cyclophilin B (CypB) is essential for JEV replication and that CypB interacts with NS4A in JEV infected cells (Kambara et al., 2011). Also, NS4A has been shown to function as an IFN antagonist (Lin et al., 2008a).

4.2.7 The NS5 protein

NS5 is the largest (105 kDa) and most highly conserved flaviviral protein that has been crystallized and characterized clearly. Like NS3, NS5 is also a multifunctional protein and contains two distinct enzymatic activities of S-adenosyl methyltransferase and RNA-dependent RNA polymerase (RdRp) (Davidson, 2009; Kim et al., 2007) that are separated by an interdomain region. The former resides in the N-terminal domain and is probably involved in performing sequential N7 and 2′ O methylations after the terminal γ phosphate is removed by NS3 during the capping process. The RdRp activity that resides in the C terminal domain of NS5 is majorly responsible for *de novo* initiation of viral RNA synthesis. NS5 can be phosphorylated by a serine/threonine kinase and this phosphorylated form has been shown to localize to the nucleus presumably via the nuclear localization signals that are found in its NS3-interacting interdomain region. In addition to its interaction with the C terminal domain of NS3 as mentioned above, NS5 may also be involved in interactions with several other nonstructural proteins that may be required for its association with other replicase components (Khromykh et al., 1996) and to facilitate virus replication, maturation and assembly. It has also been shown that NS5 has a phosphatase activity which reverses Stat-1 phosphorylation and inhibits IFN responses (Lin et al., 2006).

5. Immune responses to JEV

Both innate and adaptive immune responses are activated in response to JEV infection and have been shown to reduce serum viraemia and viral loads in infected tissues. These immunological responses – both humoral and cellular are the subject of active investigation. Humoral responses play a central role in protection against JEV. However, cell mediated immune responses contributing to this end are not fully understood. In fact, both clinical and experimental data clearly show that IgM and IgG humoral immune responses act in

conjunction with cellular immune responses to prevent virus dissemination and persistence as well as resolution of CNS infection. Ironically, the same protective immune responses can also become pathological and cause immune-mediated damage to the host.

5.1 Humoral immune response to JEV

The presence of IgM in the cerebrospinal fluid (CSF) of patients within 5-7 days appears to be a positive prognostic indicator in patients infected with JEV for the first time. Raised IgM levels are present seven days after infection with JEV in the sera and CSF of most symptomatic patients (Solomon et al., 2000). However, raised IgM levels are observed only in the serum but not in the CSF of asymptomatic patients. Maximal levels of serum IgM occur from about day 9 onwards after the onset of clinical disease. Isolation of virus has been possible only in patients who lack the primary IgM response and is usually associated with fatal outcome. Hence diagnostic tests are based on the capture of anti-JEV IgM from patient sera (Shrivastva et al., 2008; Ravi et al., 2006). This primary IgM response leads to class switching and production of IgG by 30 days after infection only in patients who survive. Neutralizing antibodies lead to inhibition of virus replication and virus spread in the initial stages while in encephalitic patients, antibody mediated inhibition of virus replication leads to the inhibition of the cytopathic effects of the virus and hence less tissue damage.

Protection from JEV challenge has been shown to be mainly antibody dependant and virus neutralizing antibodies alone are sufficient for conferring protection. Passive immunization of neutralizing monoclonal antibodies raised against E protein protects mice from lethal challenge with JEV (Zhang et al., 1989; Kimura-Kuroda and Yasui, 1988). Sub-viral particles consisting of prM in addition to E proteins were highly effective in generating a protective immune response in mice against JEV (Konishi et al., 1992b; Mason et al., 1991). The critical role of antibodies in protection against JEV has also been shown using mice lacking immunoglobulins (Pan et al., 2001) as mentioned later in the chapter. It has been shown that humanized monoclonal antibodies derived from chimpanzees protect mice from lethal JEV challenge. These protective antibodies were against different domains of E protein and exhibited high neutralizing activities against a broad spectrum of JEV genotype strains (Goncalvez et al., 2008).

Antibody responses have been observed against many nonstructural proteins but none except anti-NS1 antibodies were protective in nature. However, even these could provide only low protection from JEV infection. A recent study has shown that the *E. coli* expressed NS1 protein induces protective humoral immune response in mice during JEV infection (Lin et al., 2008b). The low level of protective immunity of NS1 was previously proposed to rely on the NS1-specific antibody-dependent complement-mediated cytolysis of the JEV-infected cells (Lin et al., 1998). However, anti NS1 antibodies can directly effect reduction in virus titres from infected cells (Krishna et al., 2009). Immunization of mice with recombinant vaccinia viruses that express various JEV gene products such as E, prM, M and NS1 have also been reported to confer protection against lethal JEV challenge in mice (Konishi et al., 1992a; Mason et al., 1991; Yasuda et al., 1990).

Enhanced neurovirulence of JEV has also been reported in mouse models by passive transfer of virus-specific antibodies. Such enhanced neurovirulence is thought to be mediated by antibody dependent enhancement (ADE) of virus infection due to the internalization of antibody-virus complexes into cells via Fcγ or complement receptors (Gould and Buckley, 1989). The role of non-neutralizing antibodies in increasing infection via antibody dependent enhancement (ADE) is still unclear although this has been thought to contribute to

the immunopathological activity of anti-flaviviral humoral responses. ADE has been observed with DNV (Kliks et al., 1989), YFV (Gould and Buckley, 1989) and other flaviviruses.
It has also been shown that oral immunization of mice with live JEV generates a brisk and protective immune response. It was also observed that more robust IgG1 antibody response against JEV occurs following oral infections compared to intraperitoneal (i.p) or subcutaneous (s.c) infections in C57BL/6 and Swiss albino mice (Ramakrishna et al., 2003). However, oral immunization of mice with JEV envelope protein that was expressed in *E. coli* also led to the generation of low neutralizing titre antibodies that were not protective. This result was ascribed to the lack of glycosylation and lack of correct folding in the *E. coli* expressed protein.
Despite the presence of comprehensive data that point out the important role of antibodies in protection, differences in the role of humoral responses in different flaviviruses are evident in studies that utilize mice lacking B cell function. In the case of WNV, mice lacking B cells are more susceptible to the disease while such mice show no change in disease severity in the case of dengue virus (Diamond et al., 2003).

5.2 Cell mediated immune response to JEV

The activation of T cell immunity during JEV infection is an area of intense scrutiny but its role is less clear. In early studies, thymus-deprived mice showed an impaired anti-JEV antibody response indicating the importance of T cell responses in generating B cell immunity to JEV infection (Mori et al., 1970). Similarly, the suppression of T cell function in spider monkeys that are normally resistant to JEV-induced disease resulted in their susceptibility (Nathanson and Cole, 1970). Life long cell mediated immunity could be conferred by passive transfer of immune spleen T cells in mice (Jia and Huang, 1983). The role of T cells in JEV infection was indicated in infection studies involving T cell deficient (nu/nu) nude mice, wherein it was observed that T cells were required for recovery (Lad et al., 1993) and protection after i.p. challenge with JEV (Miura et al., 1990). Adoptive transfer studies had shown that adult but not newborn mice were protected from JEV infection when JEV primed purified T cells were given intracerebral (i.c.) along with 10 x LD_{50} JEV. It was also shown that $CD4^+$ and $CD8^+$ T cells were necessary in protection from JEV and depletion of either population abrogated the protective ability of transferred effectors (Murali-Krishna et al., 1996). However, other reports have not supported a role for T cells in protection from JEV infection (Pan et al., 2001). In their study, neither enriched JEV primed T cells nor B cells were able to protect mice from JEV infection. Further, in their JEV challenge experiments in immunoglobulin (Ig), $CD4^+$ or $CD8^+$ T knockout mice immunized with envelope DNA vaccine, it was observed that protection was abrogated only in Ig and $CD4^+$ knockout mice but not in $CD8^+$ knockout mice suggesting that only $CD4^+$ but not $CD8^+$ T cells were required for protection. Nonetheless, $CD8^+$ T cell activation may contribute to disease pathogenesis in the case of other flaviviruses. It has been shown that mice lacking perforin and Fas/FasL expression were protected from MVEV-induced disease (Licon Luna et al., 2002). Similarly a immunopathological role for $CD8^+$ T cells has been reported for dengue virus (An et al., 2004).
JEV specific T cell responses were observed in peripheral blood mononuclear cells (PBMCs) collected from vaccinees who had received a purified formalin inactivated JEV vaccine as well as JEV patients (Konishi et al., 1995). Both $CD4^+$ and $CD8^+$ T cell subsets directed against structural proteins were observed in PBMCs isolated from vaccinees that are directed against structural proteins. In contrast, PBMCs from JEV patients showed $CD4^+$ and $CD8^+$ responses to nonstructural or C proteins.

Nonstructural proteins play a major role in the initiation of T cell responses and among the non-structural proteins, NS3 is the major target of JEV-specific cell mediated immune responses, whereas NS5 is the weakest. In another study, cytolytic $CD4^+$ T cell clones have been generated from PBMCs of individuals immunized with inactivated JEV vaccine (Aihara et al., 1998). These $CD4^+$ CTLs recognized the envelope protein and were flavivirus cross reactive in nature. The significance of such $CD4^+$ T cells in an *in vivo* context is not clear. NS3 protein (region aa193-324) is a dominant source of epitopes for both $CD4^+$ and $CD8^+$ T cells in PBMCs isolated from children belonging to a JE endemic region of India. NS3 stimulated IFN-γ production by both the $CD4^+$ and $CD8^+$ T cells indicates that a Th1 immune response to the NS3 protein may be a critical determinant of immune control during JEV infection (Kumar et al., 2004a; Kumar et al., 2004b). Recombinant JEV domain III has also been shown to elicit a Th1 type of response in Balb/c mice (Verma et al., 2009).

JEV infection using the mouse model has been reported to result in differential secretion of cytokines in the brain, spleen and sera that depended on the route of inoculation. This differential secretion could influence the overall balance between Th1 and Th2 predominance after infection and thus determine its outcome. The expression of IL-4 and IL-10 increased immediately after intracerebral (i.c.) challenge with virus and decreased later as virus load increased with concomitant increases in the expression of TNFα and IFN-γ. The levels of IL-4 and TNFα but not IL-10 and IFN-γ increased in the serum of these challenged animals (Saxena et al., 2008a). However, intraperitoneal challenge resulted in an increase in the expression of all these cytokines in the spleen (Saxena et al., 2008b). Thus, while TNFα and IFN-γ could be involved in rapid virus clearance from the periphery, they could exacerbate inflammation within the CNS.

Recent reports suggest that different flaviviruses are able to infect dendritic cells (DCs) both *in vitro* and *in vivo* and alter their phenotype and function (Cao et al., 2011). JEV is able to infect macrophages as well as dendritic cells derived from the bone marrow and spleen but it appears to have dual action on these two different antigen presenting cells of the immune system. Macrophages and DCs are antigen presenting cells that produce several cytokines such as TNFα and IL-6 upon virus infection or activation (Zhang et al., 2001). DC maturation is associated with increased antigen presentation, increased synthesis and secretion of proinflammatory cytokines such as TNFα, IL-12 and IL-6, upregulation of costimulatory molecules such as CD80, CD86 and CD40 and alteration of chemokine receptors (Kawai and Akira, 2006). They are also the principal cells that express toll-like receptor (TLR) molecules for pathogen recognition and therefore, important for innate immune responses. JEV infection of macrophages resulted in increased synthesis of cytokines and costimulatory markers but JEV infection of DCs *in vitro* led to suppressed activation, enhanced CCL2 (MCP-1) release and more prominently secretion of IL-10, a cytokine that suppresses T cell response (Pestka et al., 2004). All these mediators namely TNFα, IL-10, CCL2 and type I IFNs are crucial modulators of viral infections and have the ability to alter the polarization of Th1 and Th2 cells. Endogenous IL-10 has also been shown to decrease during JEV infection (Swarup et al., 2007a) using immuno-histochemical approaches. However, the observation that infected DCs activated Foxp3+ regulatory T (Treg) cells suggests that JEV may have the ability to suppress ongoing immune responses although this ability may not be sufficient to suppress the virus-triggered inflammatory response. Taken together with the observation that the highly efficient vaccine strain SA14-14-2 of JEV stimulated DC function and suppressed Foxp3+ Treg cells suggests an important role for dendritic cells in the JEV mediated disease process (Li et al., 2011). JEV infection has also been found to result in the

depletion of splenic $CD8\alpha^+CD11c^+$ DCs as well as suppression of MHC-I mediated antigen presentation leading to suppression of $CD8^+$ T cells (Aleyas et al., 2010).
DCs play a critical role in antigen presentation and alterations in DC function will lead to altered adaptive immune responses. However, DCs are also capable of initiating innate immune responses against viruses through TLRs that are expressed on the cell surface or intracellularly. TLRs use the pan adaptor MyD88 molecule to transduce downstream TLR signaling events. The cytokine profile induced in JEV infected DCs from wild type mice was considerably reduced in DCs that were obtained from TLR2 and TLR3 deficient mice. JEV-induced functional impairment of DCs finally led to altered $CD4^+$ and $CD8^+$ T cell function. It is hence significant that this was mediated through MyD88 dependent and independent signalling pathways implicating the involvement of TLRs in JEV pathogenesis (Aleyas et al., 2009). TLR3 plays a role in WNV entry into the CNS and has been shown to play an important immunopathological role in the WNV induced CNS disease. TLR3 deficient mice show an impaired microglial TNFα response and hence decreased primary neuronal damage and absence of encephalitis (Wang et al., 2004). All these observations suggest that JEV can modulate both adaptive and innate immunity.

6. Pathogenesis

Altered cytokine secretion profiles and the balance between proinflammatory and anti-inflammatory cytokines determine the outcome of disease processes. Immunoprotective responses to JEV infection lead to the secretion of proinflammatory cytokines in the initial phases but overproduction of these cytokines shift the balance and cause neuronal injury. JEV infection in humans is associated with elevated levels of proinflammatory mediators such as IFNα, TNFα, MIF, IL-8, IL-6, (CCL5) RANTES, Cox-2, IL-1β and (CCL2) MCP-1 (Ghoshal et al., 2007; Burke and Morill, 1987) in the CSF and/or serum. Increasing levels of such cytokines in the serum and CSF of JEV patients have been correlated with higher mortality rates (Ravi et al., 1997).
Activation of CNS cells such as microglia and astrocytes is a major histological feature of JEV infection both in animals and humans infected with JEV, MVE and WNV. JEV is a neurotropic virus and infects relatively immature neurons (Kimura-Kuroda et al., 1993), astrocytes and microglial cells which might serve as a reservoir for the virus (Thongtan et al., 2010). Several studies report the interaction of JEV with microglia and astrocytes leading to production of chemokines, inflammatory mediators and neuronal degeneration. The chemokines, CCL5 and CCL2 that are produced from JEV infected cells (Das et al., 2009a; Chen et al., 2004) result in the extravasation of activated T cells and macrophages into the brain and leads to the stimulation of microglial cells within the CNS. IL-6, MCP-1 and RANTES promote leukocyte infiltration into the brain while IP10 production by infected astrocytes leads to infiltration of NK cells and monocytes (Bhowmick et al., 2007).
Neuronal degeneration as a consequence of JEV infection may be brought about directly or indirectly. Direct effects of JEV within neurons are due to virus replication leading to cytopathicity and cell death while indirect effects are due to the action of inflammatory mediators that are produced as a result of virus infection (Ghosh and Basu, 2009). Cytotoxic effects can be the result of oxidative stress caused by the overproduction of reactive oxygen species (ROS), free radical derivatives of molecular oxygen and hydrogen peroxide. JEV has been shown to activate the ROS mediated pathway (Lin et al., 2004b) and JEV infection of primary neurons/glia, mixed glia, microglia and astrocytes results in the stimulation of

RANTES production (Chen et al., 2004). TNFα and IL-1 released during the inflammatory processes occurring in the JEV infected CNS play an important role in the induction of RANTES gene expression. These responses along with other proinflammatory cytokines increase upon JEV infection of cells within the CNS and lead to uncontrolled microglial activation and neuronal cell death. Although activated astroglial cells have the ability to neutralize the toxic effects of free radicals, they may be unable to deal with the uncontrolled inflammatory effects of JEV infection (Ghosh and Basu, 2009). In addition to the triggering of an inflammatory cascade, JEV has been shown to inhibit the proliferation of neural progenitor stem cells and thus block the replenishment of damaged neural cells which could be one of the reasons for the sequelae that are observed in surviving JEV patients. Nitric oxide (NO) is also another antiviral factor secreted from the infiltrating leukocytes and blocks virus replication (Lin et al., 1997) but the high levels of NO produced cause oxidative bystander damage leading to further neuronal damage. NO plays an important role in other flaviviral infections as well and has been reviewed elsewhere (King et al., 2007).

Programmed cell death (PCD) or apoptosis is a prominent consequence of flaviviral replication within infected cells and the ensuing release of inflammatory mediators such as TNFα (Ghosh and Basu, 2009). JEV infection triggers perturbation of ER homeostasis and the unfolded protein response, thus accelerating ER stress-induced apoptosis (Liao et al., 1997). Apoptosis can also be mediated by the virus induced release of ROS which is an antiviral defense response. JEV infection has been shown to cause apoptosis of neuroblastoma cells and activates caspase 8 and 9 in a FADD-independent and mitochondrion-dependent manner (Tsao et al., 2008). JEV non structural protein NS3 along with NS2B expression enhances apoptosis via caspase-3 activation as well as decreased mitochondrial membrane potential in human medulloblastoma cells (Yang et al., 2009).

Many of the observations made above have been supported by the results obtained recently from genomic expression profiling. Whole genomic expression profiling by cDNA microarray of JEV infected mouse brain cerebral cortex (Gupta and Rao, 2011) revealed the activation of several genes participating in immune response, inflammatory response, cell adhesion, defense response, proteolysis, endocytosis and chemokine signaling. Several other genes involved with the cell cycle, endocytosis, leukotriene metabolism, signal transduction, transcription, cell adhesion and apoptosis were downregulated during the course of disease. Significantly, genes encoding caspases 1 and 5 characteristic of the inflammasome complex were upregulated supporting the occurrence of inflammation during JEV infection. Although the exact manner in which the plethora of upregulated genes play a role in JEV mediated disease is still to be worked out, it is significant that many lectin receptors, TLR 2 and 3, OAS family, guanylate nucleotide binding proteins and TRIM family of proteins are among those that are altered. In general, these results corroborate the expression profiles that have been reported for WNV (Koh and Ng, 2005; Venter et al., 2005).

7. Interferons, MHC and NF-κB mediated regulation

IFNs play a major role in viral infections and the importance of type-I and type-II IFN in controlling viral infection was clearly demonstrated in mice which are unresponsive to either IFN-α/β or IFN-γ (Muller et al., 1994). Type-I IFN mediated immune response is the main innate immune mechanism of the host against viral infection. IFNs are one of the best characterized components of the immune system. They act in paracrine fashion to induce gene expression through the engagement of cell surface IFN receptors and by activating the

Jak-Stat signaling pathway. The coordinated activities of multiple cellular transcription factors such as IRFs, NF-κB, and c-Jun/ATF-2 are responsible for the induction of type-1 IFNs during viral infection (Doly et al., 1998; Wathelet et al., 1998). During JEV infection, both NF-κB and interferon regulatory factor-3 (IRF3) mediated signaling events are involved in the induction of IFN-β (Chang et al., 2006). WNV infection has been shown to induce IFN-β and several IFN-stimulated genes (ISG) late in the infection of cultured cells due to the delayed activation of IRF-3 (Fredericksen et al., 2004). High levels of IFN-α have been detected in patients with dengue fever or dengue hemorrhagic fever (Kurane et al., 1993). IFN-α activity was also detected in the plasma and cerebrospinal fluid specimens from patients infected with JEV (Winter et al., 2004; Burke and Morill, 1987). Type-I IFNs are produced by most cell types soon after viral infection whereas, IFN-γ is produced by a restricted set of cells of the immune system such as NK cells, $CD4^+$ T helper 1 (Th1) cells and CTLs. Although IFN-γ may play a protective role, it has been shown that lethality was less severe in WNV infected IFN-γ knockout mice suggesting that IFN-γ may be a contributory factor in the establishment of immunopathology (King et al., 2007).

Pretreatment of cell cultures with IFN-α and IFN-β inhibits the replication of flaviviruses including JEV, YFV and WNV (Ajariyakhajorn et al., 2005; Samuel and Diamond, 2005; Scherbik et al., 2007) However, the use of IFN-α in clinical trials for the treatment of JEV infection has not been successful (Solomon et al., 2003). This may be because many flaviviruses such as JEV, WNV and KUNV have developed different strategies to overcome the antiviral activities of type-I IFNs (Liu et al., 2005; Munoz-Jordan et al., 2005; Guo et al., 2005; Lin et al., 2004a; Munoz-Jordan et al., 2003). NS2A of WNV prevents the translocation of Stat1 and Stat2 into the nucleus by preventing their phosphorylation (Liu et al., 2006a). JEV is known to escape from the action of IFNs by preventing both Tyk2 tyrosine phosphorylation as well as Stat activation, thereby blocking the IFN stimulated Jak-Stat signaling (Lin et al., 2004a). This inhibition of Tyk2 tyrosine phosphorylation and Stat activation is mediated by the nonstructural protein NS5 of JEV (Lin et al., 2006). It has recently been shown that JEV encoded NS4A is able to block the phosphorylation of Stat1 and Stat2 but not Tyk2 activation in response to IFN-α/β (Lin et al., 2008a). In addition to IFNs, ISGs 49, 54 and 56 as well as the double stranded RNA activated protein kinase (PKR) and 2′,5′-oligoadenylate synthetase may play important roles in flavivirus infections (King et al., 2007).

NF-κB induced transcriptional activation plays an important role in the production of type-I IFNs during viral infection and the consequent initiation of antiviral immune responses. Many viruses have evolved different strategies either to disrupt or activate NF-κB signaling and modulate host immune response with the help of viral proteins. The NF-κB activation by viruses such as HIV-1, HTLV-1, HHV8, EBV and Influenza virus serves different functions such as to promote viral replication, to prevent virus-induced apoptosis as well as to modulate cellular apoptosis and growth (Hiscott et al., 2006; Hiscott et al., 2001). It has been shown that both JEV and WNV infections induce NF-κB activation (Kesson and King, 2001; Liao et al., 2001).

There are 2 different pathways of NF-κB activation, the classical or canonical pathway and the alternate or noncanonical pathway. The NF-κB factor is a dimer composed of different Rel proteins and is retained in the cytoplasm of a resting cell by three inhibitor proteins, IκBα, IκBβ and IκBε. In the canonical pathway, stimulus-responsive phosphorylation of the IκBs by IκB kinase (IKK) complex leads to their degradation to allow for nuclear translocation of the NF-κB dimers. The IKK complex is composed of two catalytic subunits, IKK1 (IKKα), and IKK2 (IKKβ) and the regulatory subunit IKK3 (NEMO). IKK1 kinase is

dispensable for the canonical pathway of NF-κB activation (Scheidereit, 2006). However, the noncanonical pathway critically depends on IKK1 activity for NF-κB signaling. Hence mutant mouse embryonic fibroblasts lacking in any one of these kinases or the Rel counterparts have been used to dissect the roles of the canonical and noncanonical pathways (Basak and Hoffmann, 2008). Both the classical and the alternate NF-κB signaling pathways play important but distinct roles in the functioning of the immune system. The classical pathway is largely responsible for the regulation of inflammation as well as the control of proliferation and apoptosis of lymphoid cells during the immune response. In contrast, the alternate pathway is associated with the development of lymphoid organs that ensure the mounting of an effective immune response. JEV infection also activates NF-κB in glial cells and primary mouse brain astrocytes (Abraham et al., 2008; Liao et al., 2001). Using IKK1$^{-/-}$, IKK2$^{-/-}$, IKK3$^{-/-}$ and IKK1$^{-/-}$IKK2$^{-/-}$ double mutant as well as RelA$^{-/-}$cRel$^{-/-}$p50$^{-/-}$ triple mutant mouse embryonic fibroblasts infected with JEV, it has been shown that JEV activates the classical pathway of NF-κB activation in a IKK2- and IKK3- but not IKK1-dependent manner that involved RelA and p50 complexes. NF-κB dependent and independent mechanisms also critically determined type I IFN induction in JEV infected MEFs (Abraham et al., 2010).

In addition to its involvement in immune responses, apoptosis and the cell cycle (Bonizzi and Karin, 2004), NF-κB has been shown to play a major role in flavivirus-mediated induction of MHC-I (Cheng et al., 2004). Both NF-κB dependent and independent mechanisms regulate WNV-induced MHC-I expression on infected cells. MHC molecules play an important role in host responses to viral infection and the consequences of virus infection on the expression of MHC molecules are varied. Many viruses such as HIV, MCMV, HCMV, AdV and EBV are known to decrease the expression of MHC molecules upon infection (Lilley and Ploegh, 2005). On the other hand, WNV induces the cell surface expression of both MHC-I and MHC-II molecules (Kesson et al., 2002) and the induced MHC-II was functionally intact in mouse brain astrocytes since it was associated with better recognition by a class II MHC reactive T cell line (Liu et al., 1989). In contrast to WNV, JEV infection of primary mouse brain astrocytes induces MHC-I but is unable to induce MHC-II. JEV-induced MHC-I expression was also associated with induced transcription of molecules involved in antigen processing and presentation such as *Tap1, Tap2, Tapasin, Lmp2, Lmp7* and *Lmp10* (Abraham and Manjunath, 2006). The absence of MHC-II induction during JEV infection could be important because it may lead to the initiation of an immune response which is different from other flaviviral infections that induce the expression of MHC-II molecules. Again, in contrast to WNV, the use of knockout MEFs showed that JEV-mediated induction of classical MHC-I molecules remained unaffected in NF-κB defective cells but was completely dependent on type I IFNs (Abraham et al., 2010).

In contrast to classical MHC molecules, the nonclassical MHC molecules do not belong to a single group of structurally and functionally homologous proteins and normally have lower cell surface expression. They play an important role in bridging adaptive and innate immune responses. Nonclassical MHC-I family members include gene products from HLA-E, F, G and H in humans and H2-Q, H2-T and H2-M regions in mice (Shawar et al., 1994). JEV infection of primary mouse brain astrocytes induced the expression of nonclassical MHC-I or class Ib molecules in addition to the induction of classical MHC-I molecules (Abraham and Manjunath, 2006). Given the distinct regulations of the classical and nonclassical MHC-I molecules, it was of interest to evaluate the role of the NF-κB pathway in nonclassical MHC-I gene expression.

We analyzed the role of NF-κB mediated signaling in the induction of nonclassical MHC-I molecules during JEV infection. WT, IKK1$^{-/-}$, IKK2$^{-/-}$ and IKK3$^{-/-}$ MEFs were harvested at different time intervals p.i. and expression of nonclassical MHC-I molecules was analyzed by semi quantitative RT-PCR analysis. As shown in Figure 2A, JEV infection induced the transcription of Qa-1, T10 and Qb1 in IKK1$^{-/-}$, IKK2$^{-/-}$ and IKK3$^{-/-}$ MEFs. To further validate this observation that the induction of nonclassical MHC-I molecules during JEV infection occurs independent of NF-κB activation, IKK1$^{-/-}$IKK2$^{-/-}$ double mutant MEFs were harvested at different time intervals p.i. and expression of nonclassical MHC-I molecules was analyzed by semiquantitative RT-PCR. As shown in Figure 2B, JEV infection induced the transcription of Qa-1 and Qb1 in IKK1$^{-/-}$IKK2$^{-/-}$ MEFs confirming our results that JEV-induced expression of Qa-1 and Qb1 occurs independent of NF-κB activation. The role of type-I IFNs in the induction of nonclassical MHC-I molecules during JEV infection was then determined. IFNAR$^{-/-}$ and IFNGR$^{-/-}$ MEFs were harvested at different time intervals p.i. and the expression of Qa-1, Qb1 and T10 was analyzed by RT-PCR analysis as above. As shown in Figure 2B, our RT-PCR results demonstrated that JEV-induced expression of nonclassical MHC-I molecules, Qa-1 and Qb1 was completely abrogated in IFNAR$^{-/-}$ MEFs but not in IFNGR$^{-/-}$ MEFs. In contrast, the expression of T10 was not altered in IFNAR$^{-/-}$ and IFNGR$^{-/-}$ MEFs. Thus, our results clearly show that JEV-induced expression of Qb1 and Qa-1 was dependent on type-I IFN mediated signaling. In contrast, the induction of T-10 was independent of type-I IFN mediated signaling.

The functional role of the induced nonclassical molecule T10 in the activation of γδ T cells was further analyzed. JEV infected cells were added to a cloned γδ T cell clone called G8 that responds by secretion of IL-2 upon specific recognition of the T10 molecule on the surface of cells. The G8 clone was kindly provided by Dr Chien, Stanford university school of medicine, USA. As shown in Fig 2C, exposure of G8 to JEV infected H-6 cells resulted in IL-2 secretion suggesting that the induced T10 on H6 could be functional. However, the relevance of this observation in the larger *in vivo* context is still unclear.

The involvement of γδ T cells has been shown in the case of WNV but no information is available with JEV. Mice deficient in TCRδ (TCRδ$^{-/-}$) were found to be more susceptible to WNV infection and exhibited higher viral titres in blood and lymph organs (Wang et al., 2003). A higher IFN-γ production and enhanced perforin expression in splenic T cells in WT mice as compared to TCRδ$^{-/-}$ mice was proposed to be responsible for higher survival of WT mice in WNV infection. In a recent report, it has been shown that WNV is capable of infecting γδ T-cells which leads to maturation of dendritic cells by production of IFN-γ, TNF-α and IL-6, thus facilitating adaptive immune responses as well (Fang et al., 2010).

The avidity of T cell-target interactions and the threshold for T cell activation is the result of both specific (MHC-I associated) as well as nonspecific interactions due to adhesion molecules such as ICAM-1 (Lebedeva et al., 2005). Hence, virus-induced alterations in ICAM-1 and/or VCAM-1 expression can lead to alterations not only in T cell activation but also in the leukocyte subsets attaching to the infected cells and consequently altering host cell-virus interactions. In addition to inhibition of IFN signaling, flaviviruses have been shown to modulate the expression of MHC and adhesion molecules such as VCAM and ICAM on the cell surface. Using endothelial cells, it has also been shown that flaviviruses such as WNV increases the cell surface expression of adhesion molecules such as ICAM-1, VCAM-1 as well as E-selectin. These changes were mediated by WNV in a NF-κB dependent but IFN and TNF independent manner (Shen et al., 1997).

We have reported that the cell surface expression of adhesion molecules ICAM-1 and VCAM-1 was significantly induced on primary astrocyte cultures but not hepatoma cells, L929 and 3T3 fibroblasts. Hence we observed that the JEV induced the cell surface expression of adhesion molecules does not occur uniformly on all cell types (Abraham et al., 2008). Since NF-κB transcriptionally controls the expression of ICAM-1 and VCAM-1, we further analyzed the role of NF-κB in the induction of adhesion molecules by JEV. Only VCAM-1 but not ICAM-1 was induced by JEV on mouse embryonic fibroblasts (MEFs) and knockout MEFs are the cell types that are generally utilized for analyzing the role of this transcriptional factor in gene induction studies. Hence WT (wild type), IKK1$^{-/-}$, IKK2$^{-/-}$ and IKK3$^{-/-}$ MEFs were harvested at different time intervals p.i. and expression of VCAM-1 was analyzed by flow cytometry. As shown in Figure 3, JEV infection induced the cell surface expression of VCAM-1 in IKK1$^{-/-}$MEFs but not in IKK2$^{-/-}$ and IKK3$^{-/-}$ MEFs. The basal level of expression of VCAM-1 was high in IKK2$^{-/-}$ and IKK3$^{-/-}$ MEFs. We also analyzed the status of VCAM-1 in IKK1$^{-/-}$IKK2$^{-/-}$ double knockout and RelA$^{-/-}$cRel$^{-/-}$p50$^{-/-}$triple knockout MEFs upon JEV infection by flow cytometry. As shown in Figure 3, JEV-induced expression of VCAM-1 was completely abrogated in both mutants. The basal level of expression of VCAM-1 was high in IKK1$^{-/-}$IKK2$^{-/-}$ MEFs whereas, it was low in RelA$^{-/-}$cRel$^{-/-}$p50$^{-/-}$ MEFs. This lack of induction of VCAM-1 in RelA$^{-/-}$cRel$^{-/-}$p50$^{-/-}$ MEFs indicates that the canonical pathway of NF-κB mediated signaling events are involved in the induction of VCAM-1 during JEV infection.

To determine whether JEV infection induces the expression of VCAM-1 molecules on MEFs deficient in IFN signaling, WT, IFNAR$^{-/-}$ and IFNGR$^{-/-}$ MEFs were harvested at different time intervals p.i. and the expression of VCAM-1 was analyzed by flow cytometry. As shown in the Figure 3, there was no induction of VCAM-1 on both IFNAR$^{-/-}$ and IFNGR$^{-/-}$ MEFs during JEV infection. All these results indicated that both NF-κB and type-I IFNs play a major role in the induction of VCAM-1 during JEV infection.

It has been reported that the induction of VCAM-1 molecules in response to TNF-α treatment is mediated by the coordinated activity of NF-κB and IRF-1 (Neish et al., 1995). It has also been reported that the Concanavalin A (Con A) induced expression of VCAM-1 is reduced in IRF-1$^{-/-}$ hepatic cells as compared to that of WT cells (Jaruga et al., 2004). These results point to the importance of IRF-1 in the induction of VCAM-1 molecules. However, to our surprise there was no increase of VCAM-1 on JEV infected IFNAR$^{-/-}$ MEFs but it has also been reported that IL-6 mediated induction of IRF-1 was reduced in IFNAR$^{-/-}$ MEFs as compared to that of WT MEFs (Mitani et al., 2001). Hence, we believe that the defective activity of IRF-1 in IFNAR$^{-/-}$ MEFs may be responsible for the lack of induction of VCAM-1 on IFNAR$^{-/-}$ MEFs upon JEV infection. This brings forth the possibility that even during JEV infection, the coordinated activity of both NF-κB and IRF-1 might be responsible for the induction of VCAM-1. At the same time there is one report which shows that IRF-1 is not activated upon JEV infection of human A549 cells (Chang et al., 2006) but the effects of JEV infection could be different on human cells. The mechanism of induction of ICAM-1 during JEV infection could not be characterized using knockout MEFs because ICAM-1 was induced only in astrocytes but not in MEFs.

8. Vaccines and viral inhibitors

Given the importance of flavivirus mediated encephalitis worldwide, it is not surprising that considerable efforts are underway to develop several antiviral strategies against these

viruses. These efforts include the development of antiviral drugs that inhibit not only the virus and its interactions with the host but also the development of vaccines that are aimed at strengthening antiviral immune responses as well as mosquito vector control measures to stop the spread of virus.The current vaccines that are under study are the inactivated mouse brain derived vaccine, the inactivated cell culture vaccines, the live attenuated vaccine, and the chimeric yellow fever/JE virus vaccine.

The BIKEN (JE-VAX) vaccine is derived from formalin inactivated JEV infected mouse brain and a crude inactivated version was tested in Japan as early as 1930s. The currently available version was in use from 1966. Mouse brain derived inactivated JE vaccines are based on genotype-III strains Nakayama or Beijing-1. It is prepared by clarification of Nakayama or Beijing JEV infected mouse brain suspensions by low speed centrifugation, followed by formalin inactivation, ultrafiltration, ammonium sulphate precipitation, ultracentrifugation through a 40% sucrose density gradient and dialysis (Takaku et al., 1968; Oya, 1988). This vaccine has been found to induce both JE specific and flavivirus cross reactive HLA restricted $CD4^+$ cytotoxic T cells directed against the envelope protein of the virus (Aihara et al., 1998). Vaccinated individuals exposed to infection with JEV have both memory B- and T-cell and CTL responses to the envelope protein (Aihara et al., 1998; Konishi et al., 1995). Adverse reactions have also been observed in field trials with this vaccine. These include injection site reactions (erthema, swelling, tenderness) in approximately 20% volunteers and systemic side effects (fever, headache, malaise, rashes, chills, dizziness, myaglia, nausea, vomiting and abdominal pain) in 5-10% of volunteers (Andersen and Ronne, 1991; Ruff et al., 1991; Poland et al., 1990; Hoke et al., 1988). This vaccine finds limited use in developing countries because of its high cost and has been discontinued from 2005 (Tauber et al., 2007).

Effective formalin Inactivated cell culture derived vaccine was first produced and used in China since 1960s. This was prepared from JEV infected primary hamster kidney (PHK) cells (Tsai et al., 1999). The inactivated Beijing-3 virus produced from PHK cells is the P-3 vaccine. Concerns about the presence of animal proteins in this vaccine led to development of cell culture vaccine from Vero cells infected with SA14-14-2 strain of JEV that has been adapted to PDK-8 (primary dog kidney cells). Its protective efficacy was tested in mice and phase I clinical trials (Srivastava et al., 2001). Similarly, another vaccine was derived from Vero cells infected with the Beijing strain of JEV. Phase I clinical trial of this Vero cell culture derived vaccine in 269 adult males identified a low rate of mild adverse events and greater or equivalent seroconversion rates compared to mouse brain derived vaccine (Kuzuhara et al., 2003; Sugawara et al., 2002). A vero cell-derived inactivated JE vaccine containing the purified, inactivated JEV strain SA14-14-2 with aluminum hydroxide as adjuvant has passed the Phase III randomized controlled trial (Tauber et al., 2007). A new inactivated vaccine licensed in US, Europe and Australia is also prepared from SA14-14-2 grown in Vero cells and is called IC-51 (IXIARO).

Another important vaccine is the live attenuated SA14-14-2 vaccine, which is derived via extensive passage series in primary hamster kidney cells, followed by UV irradiation, plaque purification and passage in hamsters and suckling mice (Ni et al., 1994; Aihara et al., 1991). SA14-14-2 was licensed in china in 1988 and provides 80-96% protection after a single dose and further studies in China have shown 97.5% efficacy after 2 doses 1 year apart (Tandan et al., 2007; Hennessy et al., 1996; Xin et al., 1988). Seroconversion of 99-100% and neutralizing antibody titres of 1:50 to 1:150 has been observed in field trials with this vaccine in China and South Korea (Tsai, 2000; Sohn et al., 1999; Tsai et al., 1999). Very few side effects were observed. It is now being used in Indian, Nepal and Sri Lanka.

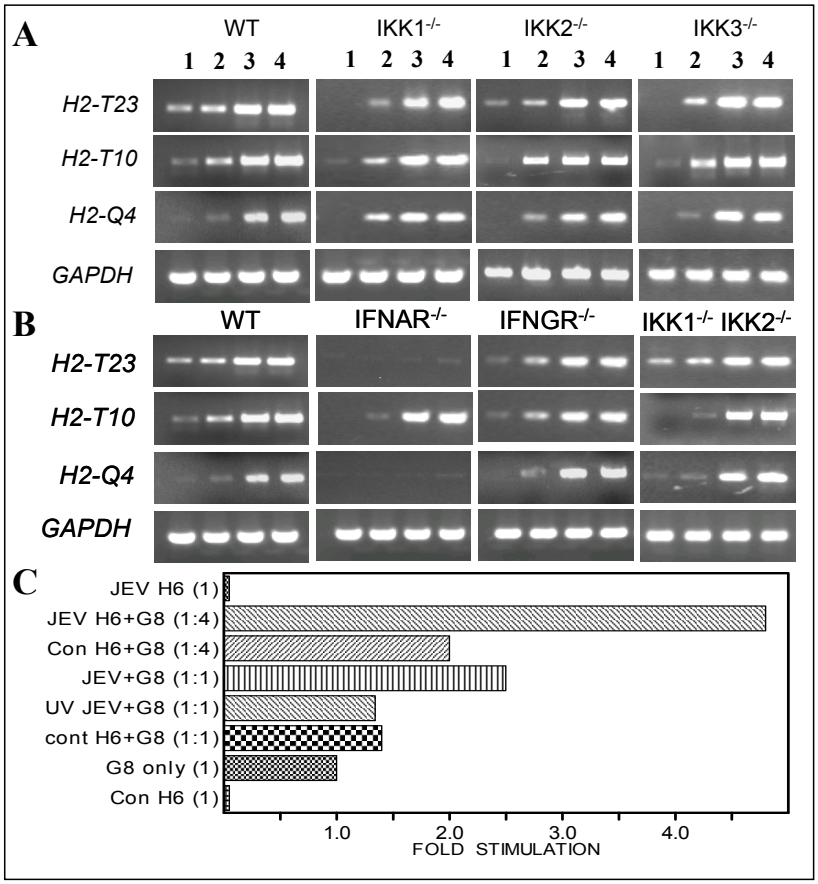

Fig. 2. JEV infection and expression of nonclassical MHC-I molecules in wild type (WT) and mutant MEFs. Panel A represents the semi quantitative RT-PCR analysis carried out for 35 cycles with uninfected and JEV infected WT, IKK1$^{-/-}$, IKK2$^{-/-}$ and IKK3$^{-/-}$ MEFs. Panel B represents the semi quantitative RT-PCR analysis carried out for 35 cycles with uninfected and JEV infected WT, IFNAR$^{-/-}$, IFNGR$^{-/-}$ and IKK1$^{-/-}$IKK2$^{-/-}$ double mutant MEFs. Lane 1 represents the result obtained from cells that were mock infected for 36 hr while lanes 2-4 represent results obtained from cells infected for 12, 24 and 36 hr. Protocols, procedures and gene specific primers used are published elsewhere (Abraham *et al*., 2010). Panel C represents IL-2 secretion from G8, γδ T cell clone upon activation with JEV infected H6 hepatoma cells. G8 cells were cultured for 24 hr alone (G8 only) or with H6 cells (Cont H6+G8), JEV infected H6 (JEV H6+G8) and H6 infected with UV inactivated JEV (UV JEV H6+G8). Uninfected H6 (Con H6) and infected H6 cultures were also cultured separately and the cell culture supernatants collected after 24 hr of stimulation were evaluated for IL-2 secretion by ELISA (BD biosciences). Values in parenthesis represent the ratios of G8:H6 cells used when 10^3 H6 cells were used for all cultures. Data from one of two different experiments are represented as fold stimulation relative to the IL-2 secretion from G8 cultured alone for 24 hr. The G8 clone was obtained as a kind gift from Dr. YH Chien, Stanford University School of Medicine, California, USA (Crowley *et al*., 1997)

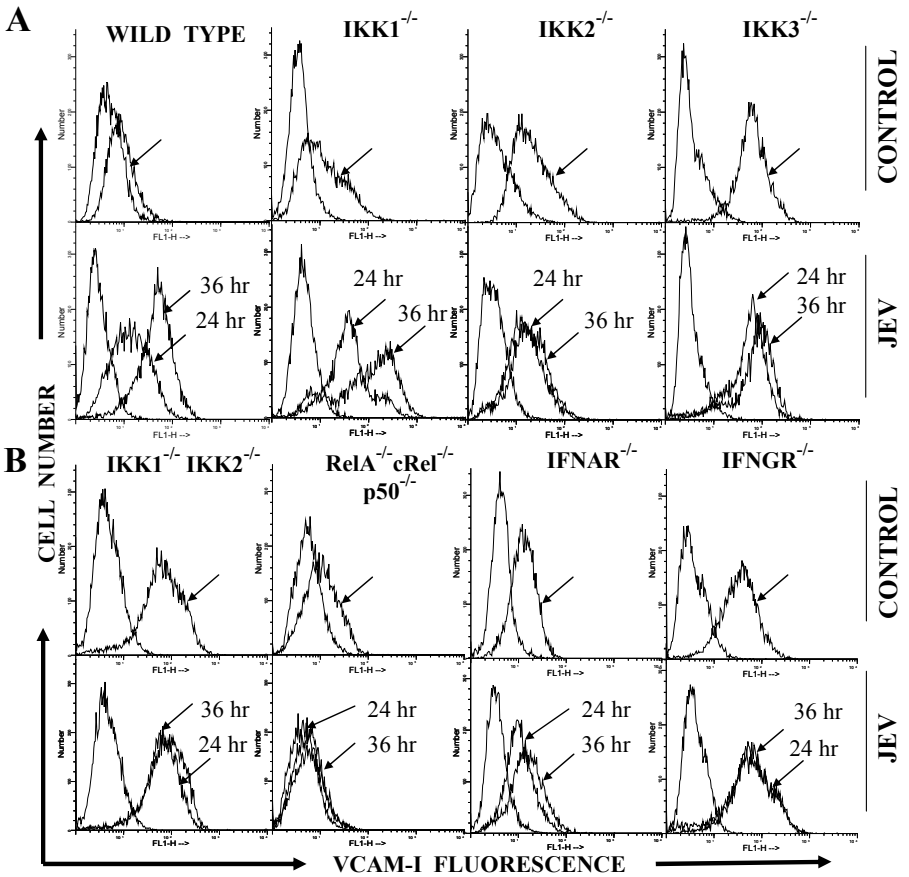

Fig. 3. JEV infection and expression of VCAM-1 on Wild type and knockout mouse embryo fibroblasts (MEFs) defective in NF-κB and interferon signaling. Wild type and knockout MEFs were infected with JEV for 24 and 36 hr and the cell surface expression of VCAM-1 was analyzed by flow cytometry. Panel A: As indicated, the upper panel (CONTROL) represents uninfected cells while the lower panel (JEV) represents infected WT, IKK1$^{-/-}$, IKK2$^{-/-}$ and IKK3$^{-/-}$ cells. Panel B: As indicated, the upper panel (CONTROL) represents uninfected cells while the lower panel (JEV) represents infected IKK1$^{-/-}$IKK2$^{-/-}$ double mutant, RelA$^{-/-}$cRel$^{-/-}$p50$^{-/-}$ triple mutant, IFNAR$^{-/-}$ and IFNGR$^{-/-}$ cells. Unmarked histograms represent staining with FITC conjugated secondary antibody alone in both panels while arrow-marked histograms in the upper panel indicate staining with unlabeled primary antibody (clone 429; anti CD 106) followed by FITC conjugated secondary antibody. They represent basal VCAM-1 expression in uninfected cells. In the lower panel, the arrows represent 24 and 36 hr post infection. Protocols, procedures and nomenclature followed are published elsewhere (Abraham *et al*., 2010)

A chimeric yellow fever/JE virus vaccine was constructed by Chambers *et al*. (1999) by substitution of prM and E genes from the highly attenuated YFV strain (strain YF17D) with that of JEV. Studies in mice and monkey models have shown that this chimeric vaccine was

highly immunogenic and protects the animals against lethal JEV i.c. challenge (Monath et al., 2000; Guirakhoo et al., 1999; Monath et al., 1999). Phase I and II clinical trials of the vaccine have been carried out and it was found to be well tolerated and induced neutralizing antibodies in 100% of naïve and yellow fever virus immune participants. In both trials it was found that a single dose of the chimeric vaccine was sufficient to elicit high titre of neutralizing antibodies (Monath et al., 2003; Monath et al., 2002). Although this vaccine was developed using genotype III virus, it stimulates protective antibodies against other genotypes as well (Beasley et al., 2004).

In addition to the development of the vaccines mentioned above, numerous studies have utilized the importance of protective epitopes in the E and NS1 protein as the basis for development of subunit vaccines to JEV. These attempts show varying degrees of protection in mouse models and use three different approaches using 1) direct protein immunization, 2) recombinant pox viruses and 3) recombinant DNA. These are discussed below:

Direct protein immunizations

These studies have evaluated different mouse model systems to evaluate the protection offered after immunization with JEV antigens in the form of recombinant subviral particles produced from cell lines, synthetic and CNBr cleaved fragments of E protein or different JEV antigens isolated from different expression systems either in the crude or purified forms.

It is known that non-infectious extracellular particles (EP) containing the M and E proteins without the nucleocapsid are secreted from BHK and Vero cells infected with a JEV prM-E-NS1-NS2A gene recombinant vaccinia virus (Mason et al., 1991). These EPs have been thought to be a promising candidate for a second generation JE subunit vaccine. Mice immunized with EPs produced from HeLa cells infected with recombinant vaccinia viruses expressing prM and E genes of JEV showed good neutralizing antibody titres and 100% protection (Konishi et al., 1992b) as well as long lasting virus specific memory T cells in mice (Konishi et al., 1997b). Similar success in affording protection was achieved with EPs from cell lines stably transformed with plasmid DNA encoding prM and E genes of JEV (Kojima et al., 2003; Hunt et al., 2001).

The C-terminal region (aa280-414) of the E protein binds several neutralizing antibodies. Hence this region or its fragments have been used in approaches that utilized their isolation from several expression systems. A protein fragment containing the C-terminal aa319-500 of the E protein was expressed along with the N-terminal 65 amino acid residues of NS1 in *E. coli* as a fusion protein with protein A. Mice immunized with this fragment produced significant protection and good neutralizing antibody titres (Srivastava et al., 1991). Mice immunized with the C-terminal region (aa280-414) of the E protein that was overexpressed in *E. coli* alone failed to generate significant protection and neutralizing antibodies. Hence, it is possible that that the N-terminal 65 amino acids of NS1 protein could have played some role in protection against viral challenge. A 27 amino acid (aa372-399) stretch within this C-terminal E fragment expressed in *E. coli*, either as a fusion protein with protein A or GST produces neutralizing antibodies (Seif et al., 1996). Immunization of mice with the same 27 amino acid peptide expressed as fusion protein with Johnson Grass Mosaic (JGM) virus-like particles also produced neutralizing antibody titres and protection was observed upon i.c. challenge with JEV (Saini and Vrati, 2003).

Expression of JEV proteins using a different expression system such as the baculovirus system has also produced moderate protection. Significant neutralizing antibody titres and moderate protection have been shown in mice immunized with recombinant baculovirus-

infected spodoptera cells expressing either E protein or a polyprotein including prM, E, NS1, NS2A, NS2B and N-terminal 28 amino acids residues of NS3 (McCown et al., 1990).
Expression of different fragments of the envelope protein reported to bind neutralizing antibodies on the surface of *Saccharomyces cerevisiae* also led to the generation of anti-JEV antibodies. However, they were not protective in mice suggesting the importance of conformational determinants for protection (Upadhyaya and Manjunath, 2009). Immunization of mice with a crude extract of *Saccharomyces cerevisiae* expressing the E protein produced only low titre neutralizing antibodies (Fujita et al., 1987).

Recombinant Pox virus based immunizations

Recombinant vaccinia viruses can also be used to generate protective immunity to JEV. Neutralizing antibodies and significant levels of protection were generated by immunizing mice with recombinant vaccinia viruses expressing prM and E genes (Yasuda et al., 1990) as well as vaccinia viruses expressing prM-E-NS1-NS2A genes of JEV. Subsequent studies introduced prM and E genes into highly attenuated strains of vaccinia viruses such as NYVAC, ALVAC and MVA (Konishi et al., 1997a; Konishi et al., 1992a; Nam et al., 1999). These approaches in mice were then followed by testing the efficacy of recombinant vaccinia virus system in humans. Highly attenuated strains of vaccinia viruses NYVAC/ALVAC expressing prM and E genes of JEV (called as NYVAC-JE and ALVAC-JE) were well tolerated in volunteers but were more reactogenic as compared to volunteers immunized with licensed form of inactivated JEV vaccine. Studies revealed that NYVAC-JE induced neutralizing antibody responses only in vaccinia non-immunised recipients, vaccinia immunized volunteers failed to develop protective antibodies. NYVAC-JE was more effective in inducing antibody responses as compared to ALVAC-JE (Kanesa-thasan et al., 2000).

Recombinant DNA based immunizations

DNA vaccines have been successful against several viral infections. Immunization using recombinant DNA constructs expressing prM-E or NS1 alone and the efficient protection obtained in mice was first reported in 1998 (Lewis and Babiuk, 1999; Lin et al., 1998). A parallel report (Konishi et al., 1998) using mice immunized with DNA constructs expressing prM-E genes of JEV showed only low levels of neutralizing antibodies but 100% protection was observed upon lethal challenge of mice with JEV. $CD8^+$ CTL response to E protein was also induced following immunization with recombinant DNA constructs. Plasmid encoding NS1 gene alone was able to afford good protection as compared to previous studies (Konishi et al., 1992a; McCown et al., 1990). The protective efficacy of several genes of JEV (C, E, NS1, NS3 and NS5) by DNA based immunization have been evaluated in mice (Chen et al., 1999). In this study only the E gene construct afforded significant protection either by i.m. immunization or by gene gun immunization but they also did not detect neutralizing antibodies before challenge in these mice. Thus, it appears that mechanism of protection induced by recombinant DNA immunization is different as compared to other immunization systems. Neutralizing antibodies were observed to be weak or absent in another DNA based vaccine attempt (Ashok and Rangarajan, 2002). The efficacy of various routes (i.d., i.v, i.m., i.p.) of immunization with plasmid DNA encoding prM and E was tested when delivered along with colloidal gold. They found that i.v. and i.d. routes generated a stronger anti-E response as compared to other routes of immunization (Zhao et al., 2003).
The studies discussed above demonstrate the potential and efficacy of various vaccines employed to generate protective immunity to JEV. Various other antiflaviviral agents that have been studied are listed in Table 2.

Sl no	Antiviral Agent	Target	System used	Reference
1	Rana catesbeiana ribonuclease (RC-RNase)	enhances apoptosis in JEV-infected cells	BHK21 cells	(Lee et al., 2011)
2	Mycophenolic acid	immunosuppressant	Mouse *In vivo*	(Sebastian et al., 2011)
3	Peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs)	JEV 3' cyclization sequence (3'CSI)	Vero (epithelial), Neuro2A (neuronal), J774E cells (macrophage)	(Anantpadma et al., 2010)
4	Octaguanidinium dendrimer-conjugated Morpholino	3′ and 5′ UTRs	Mouse, *in vivo*	(Nazmi et al., 2010)
5	Cell-penetrating peptide (CPP)-PNA conjugates	cis-acting signals at the 5'-untranslated region (UTR), 3'-UTR, and genome cyclization motifs	BHK-21 cells	(Yoo et al., 2009)
6	Pentoxifylline	virus assembly and/or release	Cell line and mouse *in vivo* mouse	(Sebastian et al., 2009)
7	Aloe-emodin (compounds from Chinese herbal medicine)	upregulates expression of IFN-stimulated genes such as dsRNA-activated protein kinase and 2',5'-oligoisoadenylate synthase	Human HL-CZ promonocyte cells and TE-671 medulloblastoma cells	(Lin et al., 2008c)
8	Bovine lactoferrin	binding to cell surface expressed heparan sulfate	CHO cells	(Chien et al., 2008)
9	Arctigenin (a plant lignin)	antiviral, neuroprotective, anti-inflammatory, antioxidative effects	Mouse *In vivo*	(Swarup et al., 2008)
10	Minocycline	anti-inflammatory and antiapoptotic drug	Mouse *In vivo*	(Mishra and Basu, 2008)
11	N-methylisatin-beta-Thiosemicarbazone (MIBT) derivative (SCH 16)	inhibit JEV replication	BHK-21 and PS cells	(Sebastian et al., 2008)
12	Rosmarinic acid	antiviral and anti-inflammatory effects	Mouse *In vivo*	(Swarup et al., 2007b)
13	N-nonyl-deoxynojirimycin (NN-DNJ), an alkyl iminosugar derivative	inhibits ER α-glucosidases I and II	BHK-21 cells	(Wu et al., 2002)

Sl no	Antiviral Agent	Target	System used	Reference
14	Bafilomycin A1	vacuolar-type proton (V-H+) pump inhibitor	Vero cells	(Andoh et al., 1998)
15	siRNA	NS5	BHK-21 cells	(Qi et al., 2008)
16	siRNA	NS1	Vero cells	(Liu et al., 2006b)
17	siRNA	cd loop-coding sequence in domain II of the viral Envelope protein	BHK-21, vero, Neuro2A cells	(Kumar et al., 2006)
18	DNAZyme	3'-NCR	J774E murine macrophage & mouse *in vivo*	(Appaiahgari and Vrati, 2007)

Table 2. Antiviral agents for JEV

9. Conclusions

Flaviviruses have developed several strategies to counter the antiviral responses that are activated in the host. It is now apparent that they can inhibit both adaptive and innate immune responses such as antigen presentation, inhibition of complement factors and interferon. However, some of these strategies that may have evolved to enhance virus survival such as activation of transcriptional factors result in the activation of inflammatory host responses as well. This results in damage to the CNS, a tissue where replenishment and renewal of damage tissue is more difficult. Virus-induction of MHC molecules is also a host response that leads to antiviral mechanisms in the long run such as activation of CTL. It is believed that all these responses are aimed at inhibition of NK cells that may kill virus infected cells immediately after infection. However, the activation of nonclassical MHC molecules by JEV could be considered as a viral strategy to the inhibit NK cells since several nonclassical MHC molecules bind to NK receptors leading to their inhibition. The presence of inflammatory responses *in vivo* highlights its importance in influencing the differences that may occur during *ex vivo* and *in vitro* studies when compared to *in vivo* studies. Hence apart from the development of new approaches to eradicate the disease, a basic understanding of the nature by which this virus alters the balance between protective and immunopathological mechanisms triggered within the infected host is also important.

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11. References

Abraham, S. and Manjunath, R. (2006) Induction of classical and nonclassical MHC-I on mouse brain astrocytes by Japanese encephalitis virus. Virus Res 119(2), 216-20.

Abraham, S., Nagaraj, A.S., Basak, S. and Manjunath, R. (2010) Japanese encephalitis virus utilizes the canonical pathway to activate NF-kappaB but it utilizes the type I interferon pathway to induce major histocompatibility complex class I expression in mouse embryonic fibroblasts. J Virol 84(11), 5485-93.

Abraham, S., Yaddanapudi, K., Thomas, S., Damodaran, A., Ramireddy, B. and Manjunath, R. (2008) Nonclassical MHC-I and Japanese encephalitis virus infection: induction of H-2Q4, H-2T23 and H-2T10. Virus Res 133(2), 239-49.

Aihara, H., Takasaki, T., Matsutani, T., Suzuki, R. and Kurane, I. (1998) Establishment and characterization of Japanese encephalitis virus-specific, human CD4(+) T-cell clones: flavivirus cross-reactivity, protein recognition, and cytotoxic activity. J Virol 72(10), 8032-6.

Aihara, S., Rao, C.M., Yu, Y.X., Lee, T., Watanabe, K., Komiya, T., Sumiyoshi, H., Hashimoto, H. and Nomoto, A. (1991) Identification of mutations that occurred on the genome of Japanese encephalitis virus during the attenuation process. Virus Genes 5(2), 95-109.

Ajariyakhajorn, C., Mammen, M.P., Jr., Endy, T.P., Gettayacamin, M., Nisalak, A., Nimmannitya, S. and Libraty, D.H. (2005) Randomized, placebo-controlled trial of nonpegylated and pegylated forms of recombinant human alpha interferon 2a for suppression of dengue virus viremia in rhesus monkeys. Antimicrob Agents Chemother 49(11), 4508-14.

Alcon, S., Talarmin, A., Debruyne, M., Falconar, A., Deubel, V. and Flamand, M. (2002) Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J Clin Microbiol 40(2), 376-81.

Aleyas, A.G., George, J.A., Han, Y.W., Rahman, M.M., Kim, S.J., Han, S.B., Kim, B.S., Kim, K. and Eo, S.K. (2009) Functional modulation of dendritic cells and macrophages by Japanese encephalitis virus through MyD88 adaptor molecule-dependent and -independent pathways. J Immunol 183(4), 2462-74.

Aleyas, A.G., Han, Y.W., George, J.A., Kim, B., Kim, K., Lee, C.K. and Eo, S.K. (2010) Multifront assault on antigen presentation by Japanese encephalitis virus subverts CD8+ T cell responses. J Immunol 185(3), 1429-41.

Allison, S.L., Schalich, J., Stiasny, K., Mandl, C.W. and Heinz, F.X. (2001) Mutational evidence for an internal fusion peptide in flavivirus envelope protein E. J Virol 75(9), 4268-75.

An, J., Zhou, D.S., Zhang, J.L., Morida, H., Wang, J.L. and Yasui, K. (2004) Dengue-specific CD8+ T cells have both protective and pathogenic roles in dengue virus infection. Immunol Lett 95(2), 167-74.

Anantpadma, M., Stein, D.A. and Vrati, S. (2010) Inhibition of Japanese encephalitis virus replication in cultured cells and mice by a peptide-conjugated morpholino oligomer. J Antimicrob Chemother 65(5), 953-61.

Andersen, M.M. and Ronne, T. (1991) Side-effects with Japanese encephalitis vaccine. Lancet 337(8748), 1044.

Andoh, T., Kawamata, H., Umatake, M., Terasawa, K., Takegami, T. and Ochiai, H. (1998) Effect of bafilomycin A1 on the growth of Japanese encephalitis virus in Vero cells. J Neurovirol 4(6), 627-31.

Appaiahgari, M.B. and Vrati, S. (2007) DNAzyme-mediated inhibition of Japanese encephalitis virus replication in mouse brain. Mol Ther 15(9), 1593-9.

Ashok, M.S. and Rangarajan, P.N. (2002) Protective efficacy of a plasmid DNA encoding Japanese encephalitis virus envelope protein fused to tissue plasminogen activator signal sequences: studies in a murine intracerebral virus challenge model. Vaccine 20(11-12), 1563-70.

Assenberg, R., Mastrangelo, E., Walter, T.S., Verma, A., Milani, M., Owens, R.J., Stuart, D.I., Grimes, J.M. and Mancini, E.J. (2009) Crystal structure of a novel conformational state of the flavivirus NS3 protein: implications for polyprotein processing and viral replication. J Virol 83(24), 12895-906.

Basak, S. and Hoffmann, A. (2008) Crosstalk via the NF-kappaB signaling system. Cytokine Growth Factor Rev 19(3-4), 187-97.

Beasley, D.W., Li, L., Suderman, M.T., Guirakhoo, F., Trent, D.W., Monath, T.P., Shope, R.E. and Barrett, A.D. (2004) Protection against Japanese encephalitis virus strains representing four genotypes by passive transfer of sera raised against ChimeriVax-JE experimental vaccine. Vaccine 22(27-28), 3722-6.

Bhowmick, S., Duseja, R., Das, S., Appaiahgiri, M.B., Vrati, S. and Basu, A. (2007) Induction of IP-10 (CXCL10) in astrocytes following Japanese encephalitis. Neurosci Lett 414(1), 45-50.

Bollati, M., Milani, M., Mastrangelo, E., Ricagno, S., Tedeschi, G., Nonnis, S., Decroly, E., Selisko, B., de Lamballerie, X., Coutard, B., Canard, B. and Bolognesi, M. (2009) Recognition of RNA cap in the Wesselsbron virus NS5 methyltransferase domain: implications for RNA-capping mechanisms in Flavivirus. J Mol Biol 385(1), 140-52.

Bonizzi, G. and Karin, M. (2004) The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25(6), 280-8.

Brandt, W.E., Chiewslip, D., Harris, D.L. and Russell, P.K. (1970) Partial purification and characterization of a dengue virus soluble complement-fixing antigen. J Immunol 105(6), 1565-8.

Burke, D.S., Lorsomrudee, W., Leake, C.J., Hoke, C.H., Nisalak, A., Chongswasdi, V. and Laorakpongse, T. (1985) Fatal outcome in Japanese encephalitis. Am J Trop Med Hyg 34(6), 1203-10.

Burke, D.S. and Morill, J.C. (1987) Levels of interferon in the plasma and cerebrospinal fluid of patients with acute Japanese encephalitis. J Infect Dis 155(4), 797-9.

Cao, S., Li, Y., Ye, J., Yang, X., Chen, L., Liu, X. and Chen, H. (2011) Japanese encephalitis Virus wild strain infection suppresses dendritic cells maturation and function, and causes the expansion of regulatory T cells. Virol J 8, 39.

Cecilia, D. and Gould, E.A. (1991) Nucleotide changes responsible for loss of neuroinvasiveness in Japanese encephalitis virus neutralization-resistant mutants. Virology 181(1), 70-7.

Chambers, T.J., Hahn, C.S., Galler, R. and Rice, C.M. (1990) Flavivirus genome organization, expression, and replication. Annu Rev Microbiol 44, 649-88.

Chambers, T.J., Nestorowicz, A., Mason, P.W. and Rice, C.M. (1999) Yellow fever/Japanese encephalitis chimeric viruses: construction and biological properties. J Virol 73(4), 3095-101.

Chang, T.H., Liao, C.L. and Lin, Y.L. (2006) Flavivirus induces interferon-beta gene expression through a pathway involving RIG-I-dependent IRF-3 and PI3K-dependent NF-kappaB activation. Microbes Infect 8(1), 157-71.

Chavez, J.H., Silva, J.R., Amarilla, A.A. and Moraes Figueiredo, L.T. (2010) Domain III peptides from flavivirus envelope protein are useful antigens for serologic diagnosis and targets for immunization. Biologicals 38(6), 613-8.

Chen, C.J., Chen, J.H., Chen, S.Y., Liao, S.L. and Raung, S.L. (2004) Upregulation of RANTES gene expression in neuroglia by Japanese encephalitis virus infection. J Virol 78(22), 12107-19.

Chen, H.W., Pan, C.H., Liau, M.Y., Jou, R., Tsai, C.J., Wu, H.J., Lin, Y.L. and Tao, M.H. (1999) Screening of protective antigens of Japanese encephalitis virus by DNA immunization: a comparative study with conventional viral vaccines. J Virol 73(12), 10137-45.

Cheng, Y., King, N.J. and Kesson, A.M. (2004) Major histocompatibility complex class I (MHC-I) induction by West Nile virus: involvement of 2 signaling pathways in MHC-I up-regulation. J Infect Dis 189(4), 658-68.

Chia, S.C., Leung, P.S., Liao, C.P., Huang, J.H. and Lee, S.T. (2001) Fragment of Japanese encephalitis virus envelope protein produced in Escherichia coli protects mice from virus challenge. Microb Pathog 31(1), 9-19.

Chien, H.L., Liao, C.L. and Lin, Y.L. (2011) FUSE binding protein 1 interacts with untranslated regions of Japanese encephalitis virus RNA and negatively regulates viral replication. J Virol 85(10), 4698-706.

Chien, Y.J., Chen, W.J., Hsu, W.L. and Chiou, S.S. (2008) Bovine lactoferrin inhibits Japanese encephalitis virus by binding to heparan sulfate and receptor for low density lipoprotein. Virology 379(1), 143-51.

Chu, J.J. and Ng, M.L. (2004) Infectious entry of West Nile virus occurs through a clathrin-mediated endocytic pathway. J Virol 78(19), 10543-55.

Chung, K.M., Liszewski, M.K., Nybakken, G., Davis, A.E., Townsend, R.R., Fremont, D.H., Atkinson, J.P. and Diamond, M.S. (2006a) West Nile virus nonstructural protein NS1 inhibits complement activation by binding the regulatory protein factor H. Proc Natl Acad Sci U S A 103(50), 19111-6.

Chung, K.M., Nybakken, G.E., Thompson, B.S., Engle, M.J., Marri, A., Fremont, D.H. and Diamond, M.S. (2006b) Antibodies against West Nile Virus nonstructural protein NS1 prevent lethal infection through Fc gamma receptor-dependent and -independent mechanisms. J Virol 80(3), 1340-51.

Crowley, M.P., Reich, Z., Mavaddat, N., Altman, J.D. and Chien, Y. (1997) The recognition of the nonclassical major histocompatibility complex (MHC) class I molecule, T10, by the gammadelta T cell, G8. J Exp Med 185(7), 1223-30.

D.S Burke and Monath, T.P. (2001) Flaviviruses. In: D.M. Knipe and P.M. Howley (Eds), Fields Virology fourth ed., pp. 1043–1125. Vol. I. Lippincott Williams and Wilkins, Philadelphia.

D.S. Burke and Leake, C.J. (1988) Japanese encephalitis. In: T.P. Monath (Ed), The Arboviruses: Epidemiology and Ecology, pp. 63-92. Vol. III. CRC Press, Boca Raton, FL.

Das, S., Ghosh, D. and Basu, A. (2009a) Japanese encephalitis virus induce immuno-competency in neural stem/progenitor cells. PLoS One 4(12), e8134.

Das, S., Laxminarayana, S.V., Chandra, N., Ravi, V. and Desai, A. (2009b) Heat shock protein 70 on Neuro2a cells is a putative receptor for Japanese encephalitis virus. Virology 385(1), 47-57.

Davidson, A.D. (2009) Chapter 2. New insights into flavivirus nonstructural protein 5. Adv Virus Res 74, 41-101.

Davis, W.G., Blackwell, J.L., Shi, P.Y. and Brinton, M.A. (2007) Interaction between the cellular protein eEF1A and the 3'-terminal stem-loop of West Nile virus genomic RNA facilitates viral minus-strand RNA synthesis. J Virol 81(18), 10172-87.

Deng, Y.Q., Dai, J.X., Ji, G.H., Jiang, T., Wang, H.J., Yang, H.O., Tan, W.L., Liu, R., Yu, M., Ge, B.X., Zhu, Q.Y., Qin, E.D., Guo, Y.J. and Qin, C.F. (2011) A broadly flavivirus cross-neutralizing monoclonal antibody that recognizes a novel epitope within the fusion loop of E protein. PLoS One 6(1), e16059.

Dewasthaly, S., Ayachit, V.M., Sarthi, S.A. and Gore, M.M. (2001) Monoclonal antibody raised against envelope glycoprotein peptide neutralizes Japanese encephalitis virus. Arch Virol 146(7), 1427-35.

Diamond, M.S., Shrestha, B., Marri, A., Mahan, D. and Engle, M. (2003) B cells and antibody play critical roles in the immediate defense of disseminated infection by West Nile encephalitis virus. J Virol 77(4), 2578-86.

Doly, J., Civas, A., Navarro, S. and Uze, G. (1998) Type I interferons: expression and signalization. Cell Mol Life Sci 54(10), 1109-21.

Endy, T.P. and Nisalak, A. (2002) Japanese encephalitis virus: ecology and epidemiology. Curr Top Microbiol Immunol 267, 11-48.

Erlanger, T.E., Weiss, S., Keiser, J., Utzinger, J. and Wiedenmayer, K. (2009) Past, present, and future of Japanese encephalitis. Emerg Infect Dis 15(1), 1-7.

Falgout, B. and Markoff, L. (1995) Evidence that flavivirus NS1-NS2A cleavage is mediated by a membrane-bound host protease in the endoplasmic reticulum. J Virol 69(11), 7232-43.

Fan, W.F. and Mason, P.W. (1990) Membrane association and secretion of the Japanese encephalitis virus NS1 protein from cells expressing NS1 cDNA. Virology 177(2), 470-6.

Fang, H., Welte, T., Zheng, X., Chang, G.J., Holbrook, M.R., Soong, L. and Wang, T. (2010) gammadelta T cells promote the maturation of dendritic cells during West Nile virus infection. FEMS Immunol Med Microbiol 59(1), 71-80.

Fredericksen, B.L., Smith, M., Katze, M.G., Shi, P.Y. and Gale, M., Jr. (2004) The host response to West Nile Virus infection limits viral spread through the activation of the interferon regulatory factor 3 pathway. J Virol 78(14), 7737-47.

Fritz, R., Blazevic, J., Taucher, C., Pangerl, K., Heinz, F.X. and Stiasny, K. (2011) The unique transmembrane hairpin of the flavivirus fusion protein E is essential for membrane fusion. J Virol 85(9), 4377-85.

Fujita, H., Sumiyoshi, H., Mori, C., Manabe, S., Takagi, M., Yoshida, I., Morita, K., Fuke, I., Fukai, K. and Igarashi, A. (1987) Studies in the development of Japanese encephalitis vaccine: expression of virus envelope glycoprotein V3 (E) gene in yeast. Bull World Health Organ 65(3), 303-8.

Ghosh, D. and Basu, A. (2009) Japanese encephalitis-a pathological and clinical perspective. PLoS Negl Trop Dis 3(9), e437.

Ghoshal, A., Das, S., Ghosh, S., Mishra, M.K., Sharma, V., Koli, P., Sen, E. and Basu, A. (2007) Proinflammatory mediators released by activated microglia induces neuronal death in Japanese encephalitis. Glia 55(5), 483-96.

Goncalvez, A.P., Chien, C.H., Tubthong, K., Gorshkova, I., Roll, C., Donau, O., Schuck, P., Yoksan, S., Wang, S.D., Purcell, R.H. and Lai, C.J. (2008) Humanized monoclonal antibodies derived from chimpanzee Fabs protect against Japanese encephalitis virus in vitro and in vivo. J Virol 82(14), 7009-21.

Gould, E.A. and Buckley, A. (1989) Antibody-dependent enhancement of yellow fever and Japanese encephalitis virus neurovirulence. J Gen Virol 70 (Pt 6), 1605-8.

Gubler, D.J. (2002) The global emergence/resurgence of arboviral diseases as public health problems. Arch Med Res 33(4), 330-42.

Guirakhoo, F., Zhang, Z.X., Chambers, T.J., Delagrave, S., Arroyo, J., Barrett, A.D. and Monath, T.P. (1999) Immunogenicity, genetic stability, and protective efficacy of a recombinant, chimeric yellow fever-Japanese encephalitis virus (ChimeriVax-JE) as a live, attenuated vaccine candidate against Japanese encephalitis. Virology 257(2), 363-72.

Guo, J.T., Hayashi, J. and Seeger, C. (2005) West Nile virus inhibits the signal transduction pathway of alpha interferon. J Virol 79(3), 1343-50.

Gupta, N. and Rao, P.L. (2011) Transcriptomic profile of host response in Japanese encephalitis virus infection. Virol J 8, 92.

Hanna, J.N., Ritchie, S.A., Phillips, D.A., Lee, J.M., Hills, S.L., van den Hurk, A.F., Pyke, A.T., Johansen, C.A. and Mackenzie, J.S. (1999) Japanese encephalitis in north Queensland, Australia, 1998. Med J Aust 170(11), 533-6.

Heinz, F.X. and Allison, S.L. (2000) Structures and mechanisms in flavivirus fusion. Adv Virus Res 55, 231-69.

Heinz, F.X., Collet, M.S., H., P.R., Gould, E.A., Howard, C.R., Houghton, M., M., M.R.J., Rice, C.M. and J., T.H. (2000) Family *Flaviviridae*. In: M.H.V. Regenmortel, C.M. Fauquet, D.H.L. Bishop, E. Carstens, M.K. Estes, S. Lemon, J. Maniloff, M. M. A, D. McGeoch, C.R. Pringle and R.B. Wickner (Eds), Virus Taxonomy: 7th International Committie for the Taxanomy of Viruses, pp. 859-878. Academic Press, San Diego.

Hennessy, S., Liu, Z., Tsai, T.F., Strom, B.L., Wan, C.M., Liu, H.L., Wu, T.X., Yu, H.J., Liu, Q.M., Karabatsos, N., Bilker, W.B. and Halstead, S.B. (1996) Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2): a case-control study. Lancet 347(9015), 1583-6.

Hiscott, J., Kwon, H. and Genin, P. (2001) Hostile takeovers: viral appropriation of the NF-kappaB pathway. J Clin Invest 107(2), 143-51.

Hiscott, J., Nguyen, T.L., Arguello, M., Nakhaei, P. and Paz, S. (2006) Manipulation of the nuclear factor-kappaB pathway and the innate immune response by viruses. Oncogene 25(51), 6844-67.

Hoke, C.H., Nisalak, A., Sangawhipa, N., Jatanasen, S., Laorakapongse, T., Innis, B.L., Kotchasenee, S., Gingrich, J.B., Latendresse, J., Fukai, K. and et al. (1988) Protection against Japanese encephalitis by inactivated vaccines. N Engl J Med 319(10), 608-14.

Hunt, A.R., Cropp, C.B. and Chang, G.J. (2001) A recombinant particulate antigen of Japanese encephalitis virus produced in stably-transformed cells is an effective noninfectious antigen and subunit immunogen. J Virol Methods 97(1-2), 133-49.

Huy, B.V., Tu, H.C., Luan, T.V. and Lindqvist, R. (1994) Early mental and neurological sequelae after Japanese B encephalitis. Southeast Asian J Trop Med Public Health 25(3), 549-53.

Jacobs, M.G., Robinson, P.J., Bletchly, C., Mackenzie, J.M. and Young, P.R. (2000) Dengue virus nonstructural protein 1 is expressed in a glycosyl-phosphatidylinositol-linked form that is capable of signal transduction. FASEB J 14(11), 1603-10.

Jaruga, B., Hong, F., Kim, W.H. and Gao, B. (2004) IFN-gamma/STAT1 acts as a proinflammatory signal in T cell-mediated hepatitis via induction of multiple chemokines and adhesion molecules: a critical role of IRF-1. Am J Physiol Gastrointest Liver Physiol 287(5), G1044-52.

Jia, F.L. and Huang, Z.X. (1983) [Cell-mediated immunity in mice infected with Japanese encephalitis virus. II. Passive transfer of immune spleen T cells for life protection in infected mice]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 5(1), 68-9.

Kabilan, L., Rajendran, R., Arunachalam, N., Ramesh, S., Srinivasan, S., Samuel, P.P. and Dash, A.P. (2004) Japanese encephalitis in India: an overview. Indian J Pediatr 71(7), 609-15.

Kambara, H., Tani, H., Mori, Y., Abe, T., Katoh, H., Fukuhara, T., Taguwa, S., Moriishi, K. and Matsuura, Y. (2011) Involvement of cyclophilin B in the replication of Japanese encephalitis virus. Virology 412(1), 211-9.

Kanesa-thasan, N., Smucny, J.J., Hoke, C.H., Marks, D.H., Konishi, E., Kurane, I., Tang, D.B., Vaughn, D.W., Mason, P.W. and Shope, R.E. (2000) Safety and immunogenicity of NYVAC-JEV and ALVAC-JEV attenuated recombinant Japanese encephalitis virus--poxvirus vaccines in vaccinia-nonimmune and vaccinia-immune humans. Vaccine 19(4-5), 483-91.

Kawai, T. and Akira, S. (2006) Innate immune recognition of viral infection. Nat Immunol 7(2), 131-7.

Kesson, A.M., Cheng, Y. and King, N.J. (2002) Regulation of immune recognition molecules by flavivirus, West Nile. Viral Immunol 15(2), 273-83.

Kesson, A.M. and King, N.J. (2001) Transcriptional regulation of major histocompatibility complex class I by flavivirus West Nile is dependent on NF-kappaB activation. J Infect Dis 184(8), 947-54.

Khromykh, A.A., Harvey, T.J., Abedinia, M. and Westaway, E.G. (1996) Expression and purification of the seven nonstructural proteins of the flavivirus Kunjin in the E. coli and the baculovirus expression systems. J Virol Methods 61(1-2), 47-58.

Kim, J.M., Yun, S.I., Song, B.H., Hahn, Y.S., Lee, C.H., Oh, H.W. and Lee, Y.M. (2008) A single N-linked glycosylation site in the Japanese encephalitis virus prM protein is critical for cell type-specific prM protein biogenesis, virus particle release, and pathogenicity in mice. J Virol 82(16), 7846-62.

Kim, Y.G., Yoo, J.S., Kim, J.H., Kim, C.M. and Oh, J.W. (2007) Biochemical characterization of a recombinant Japanese encephalitis virus RNA-dependent RNA polymerase. BMC Mol Biol 8, 59.

Kimura-Kuroda, J., Ichikawa, M., Ogata, A., Nagashima, K. and Yasui, K. (1993) Specific tropism of Japanese encephalitis virus for developing neurons in primary rat brain culture. Arch Virol 130(3-4), 477-84.

Kimura-Kuroda, J. and Yasui, K. (1983) Topographical analysis of antigenic determinants on envelope glycoprotein V3 (E) of Japanese encephalitis virus, using monoclonal antibodies. J Virol 45(1), 124-32.

Kimura-Kuroda, J. and Yasui, K. (1988) Protection of mice against Japanese encephalitis virus by passive administration with monoclonal antibodies. J Immunol 141(10), 3606-10.

King, N.J., Getts, D.R., Getts, M.T., Rana, S., Shrestha, B. and Kesson, A.M. (2007) Immunopathology of flavivirus infections. Immunol Cell Biol 85(1), 33-42.

Kitai, Y., Kondo, T. and Konishi, E. (2011) Non-structural protein 1 (NS1) antibody-based assays to differentiate West Nile (WN) virus from Japanese encephalitis virus infections in horses: effects of WN virus NS1 antibodies induced by inactivated WN vaccine. J Virol Methods 171(1), 123-8.

Kliks, S.C., Nisalak, A., Brandt, W.E., Wahl, L. and Burke, D.S. (1989) Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. Am J Trop Med Hyg 40(4), 444-51.

Koh, W.L. and Ng, M.L. (2005) Molecular mechanisms of West Nile virus pathogenesis in brain cell. Emerg Infect Dis 11(4), 629-32.

Kojima, A., Yasuda, A., Asanuma, H., Ishikawa, T., Takamizawa, A., Yasui, K. and Kurata, T. (2003) Stable high-producer cell clone expressing virus-like particles of the Japanese encephalitis virus e protein for a second-generation subunit vaccine. J Virol 77(16), 8745-55.

Kolaskar, A.S. and Kulkarni-Kale, U. (1999) Prediction of three-dimensional structure and mapping of conformational epitopes of envelope glycoprotein of Japanese encephalitis virus. Virology 261(1), 31-42.

Konishi, E., Kurane, I., Mason, P.W., Innis, B.L. and Ennis, F.A. (1995) Japanese encephalitis virus-specific proliferative responses of human peripheral blood T lymphocytes. Am J Trop Med Hyg 53(3), 278-83.

Konishi, E., Kurane, I., Mason, P.W., Shope, R.E. and Ennis, F.A. (1997a) Poxvirus-based Japanese encephalitis vaccine candidates induce JE virus-specific CD8+ cytotoxic T lymphocytes in mice. Virology 227(2), 353-60.

Konishi, E., Pincus, S., Fonseca, B.A., Shope, R.E., Paoletti, E. and Mason, P.W. (1991) Comparison of protective immunity elicited by recombinant vaccinia viruses that synthesize E or NS1 of Japanese encephalitis virus. Virology 185(1), 401-10.

Konishi, E., Pincus, S., Paoletti, E., Laegreid, W.W., Shope, R.E. and Mason, P.W. (1992a) A highly attenuated host range-restricted vaccinia virus strain, NYVAC, encoding the prM, E, and NS1 genes of Japanese encephalitis virus prevents JEV viremia in swine. Virology 190(1), 454-8.

Konishi, E., Pincus, S., Paoletti, E., Shope, R.E., Burrage, T. and Mason, P.W. (1992b) Mice immunized with a subviral particle containing the Japanese encephalitis virus prM/M and E proteins are protected from lethal JEV infection. Virology 188(2), 714-20.

Konishi, E., Win, K.S., Kurane, I., Mason, P.W., Shope, R.E. and Ennis, F.A. (1997b) Particulate vaccine candidate for Japanese encephalitis induces long-lasting virus-specific memory T lymphocytes in mice. Vaccine 15(3), 281-6.

Konishi, E., Yamaoka, M., Khin Sane, W., Kurane, I. and Mason, P.W. (1998) Induction of protective immunity against Japanese encephalitis in mice by immunization with a plasmid encoding Japanese encephalitis virus premembrane and envelope genes. J Virol 72(6), 4925-30.

Krishna, V.D., Rangappa, M. and Satchidanandam, V. (2009) Virus-specific cytolytic antibodies to nonstructural protein 1 of Japanese encephalitis virus effect reduction of virus output from infected cells. J Virol 83(10), 4766-77.

Kuhn, R.J., Zhang, W., Rossmann, M.G., Pletnev, S.V., Corver, J., Lenches, E., Jones, C.T., Mukhopadhyay, S., Chipman, P.R., Strauss, E.G., Baker, T.S. and Strauss, J.H. (2002) Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. Cell 108(5), 717-25.

Kumar, P., Lee, S.K., Shankar, P. and Manjunath, N. (2006) A single siRNA suppresses fatal encephalitis induced by two different flaviviruses. PLoS Med 3(4), e96.

Kumar, P., Sulochana, P., Nirmala, G., Chandrashekar, R., Haridattatreya, M. and Satchidanandam, V. (2004a) Impaired T helper 1 function of nonstructural protein 3-specific T cells in Japanese patients with encephalitis with neurological sequelae. J Infect Dis 189(5), 880-91.

Kumar, P., Sulochana, P., Nirmala, G., Haridattatreya, M. and Satchidanandam, V. (2004b) Conserved amino acids 193-324 of non-structural protein 3 are a dominant source of peptide determinants for CD4+ and CD8+ T cells in a healthy Japanese encephalitis virus-endemic cohort. J Gen Virol 85(Pt 5), 1131-43.

Kumar, R., Mathur, A., Singh, K.B., Sitholey, P., Prasad, M., Shukla, R., Agarwal, S.P. and Arockiasamy, J. (1993) Clinical sequelae of Japanese encephalitis in children. Indian J Med Res 97, 9-13.

Kummerer, B.M. and Rice, C.M. (2002) Mutations in the yellow fever virus nonstructural protein NS2A selectively block production of infectious particles. J Virol 76(10), 4773-84.

Kurane, I., Innis, B.L., Nimmannitya, S., Nisalak, A., Meager, A. and Ennis, F.A. (1993) High levels of interferon alpha in the sera of children with dengue virus infection. Am J Trop Med Hyg 48(2), 222-9.

Kuzuhara, S., Nakamura, H., Hayashida, K., Obata, J., Abe, M., Sonoda, K., Nishiyama, K., Sugawara, K., Takeda, K., Honda, T., Matsui, H., Shigaki, T., Kino, Y., Mizokami, H., Tanaka, M., Mizuno, K. and Ueda, K. (2003) Non-clinical and phase I clinical trials of a Vero cell-derived inactivated Japanese encephalitis vaccine. Vaccine 21(31), 4519-26.

Lad, V.J., Gupta, A.K., Goverdhan, M.K., Ayachit, V.L., Rodrigues, J.J. and Hungund, L.V. (1993) Susceptibility of BL6 nude (congenitally athymic) mice to Japanese encephalitis virus by the peripheral route. Acta Virol 37(4), 232-40.

Lanciotti, R.S., Roehrig, J.T., Deubel, V., Smith, J., Parker, M., Steele, K., Crise, B., Volpe, K.E., Crabtree, M.B., Scherret, J.H., Hall, R.A., MacKenzie, J.S., Cropp, C.B., Panigrahy, B., Ostlund, E., Schmitt, B., Malkinson, M., Banet, C., Weissman, J., Komar, N., Savage, H.M., Stone, W., McNamara, T. and Gubler, D.J. (1999) Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 286(5448), 2333-7.

Lebedeva, T., Dustin, M.L. and Sykulev, Y. (2005) ICAM-1 co-stimulates target cells to facilitate antigen presentation. Curr Opin Immunol 17(3), 251-8.

Lee, C.J., Lin, H.R., Liao, C.L. and Lin, Y.L. (2008) Cholesterol effectively blocks entry of flavivirus. J Virol 82(13), 6470-80.

Lee, Y.H., Wei, C.W., Wang, J.J. and Chiou, C.T. (2011) Rana catesbeiana ribonuclease inhibits Japanese encephalitis virus (JEV) replication and enhances apoptosis of JEV-infected BHK-21 cells. Antiviral Res 89(3), 193-8.

Leung, J.Y., Pijlman, G.P., Kondratieva, N., Hyde, J., Mackenzie, J.M. and Khromykh, A.A. (2008) Role of nonstructural protein NS2A in flavivirus assembly. J Virol 82(10), 4731-41.

Lewis, P.J. and Babiuk, L.A. (1999) DNA vaccines: a review. Adv Virus Res 54, 129-88.

Li, W., Li, Y., Kedersha, N., Anderson, P., Emara, M., Swiderek, K.M., Moreno, G.T. and Brinton, M.A. (2002) Cell proteins TIA-1 and TIAR interact with the 3' stem-loop of

the West Nile virus complementary minus-strand RNA and facilitate virus replication. J Virol 76(23), 11989-2000.

Li, Y., Ye, J., Yang, X., Xu, M., Chen, L., Mei, L., Zhu, J., Liu, X., Chen, H. and Cao, S. (2011) Infection of mouse bone marrow-derived dendritic cells by live attenuated Japanese encephalitis virus induces cells maturation and triggers T cells activation. Vaccine 29(4), 855-62.

Liao, C.L., Lin, Y.L., Wang, J.J., Huang, Y.L., Yeh, C.T., Ma, S.H. and Chen, L.K. (1997) Effect of enforced expression of human bcl-2 on Japanese encephalitis virus-induced apoptosis in cultured cells. J Virol 71(8), 5963-71.

Liao, C.L., Lin, Y.L., Wu, B.C., Tsao, C.H., Wang, M.C., Liu, C.I., Huang, Y.L., Chen, J.H., Wang, J.P. and Chen, L.K. (2001) Salicylates inhibit flavivirus replication independently of blocking nuclear factor kappa B activation. J Virol 75(17), 7828-39.

Licon Luna, R.M., Lee, E., Mullbacher, A., Blanden, R.V., Langman, R. and Lobigs, M. (2002) Lack of both Fas ligand and perforin protects from flavivirus-mediated encephalitis in mice. J Virol 76(7), 3202-11.

Lilley, B.N. and Ploegh, H.L. (2005) Viral modulation of antigen presentation: manipulation of cellular targets in the ER and beyond. Immunol Rev 207, 126-44.

Lin, C.W., Cheng, C.W., Yang, T.C., Li, S.W., Cheng, M.H., Wan, L., Lin, Y.J., Lai, C.H., Lin, W.Y. and Kao, M.C. (2008a) Interferon antagonist function of Japanese encephalitis virus NS4A and its interaction with DEAD-box RNA helicase DDX42. Virus Res 137(1), 49-55.

Lin, C.W., Liu, K.T., Huang, H.D. and Chen, W.J. (2008b) Protective immunity of E. coli-synthesized NS1 protein of Japanese encephalitis virus. Biotechnol Lett 30(2), 205-14.

Lin, C.W., Wu, C.F., Hsiao, N.W., Chang, C.Y., Li, S.W., Wan, L., Lin, Y.J. and Lin, W.Y. (2008c) Aloe-emodin is an interferon-inducing agent with antiviral activity against Japanese encephalitis virus and enterovirus 71. Int J Antimicrob Agents 32(4), 355-9.

Lin, C.W. and Wu, S.C. (2003) A functional epitope determinant on domain III of the Japanese encephalitis virus envelope protein interacted with neutralizing-antibody combining sites. J Virol 77(4), 2600-6.

Lin, R.J., Chang, B.L., Yu, H.P., Liao, C.L. and Lin, Y.L. (2006) Blocking of interferon-induced Jak-Stat signaling by Japanese encephalitis virus NS5 through a protein tyrosine phosphatase-mediated mechanism. J Virol 80(12), 5908-18.

Lin, R.J., Liao, C.L., Lin, E. and Lin, Y.L. (2004a) Blocking of the alpha interferon-induced Jak-Stat signaling pathway by Japanese encephalitis virus infection. J Virol 78(17), 9285-94.

Lin, R.J., Liao, C.L. and Lin, Y.L. (2004b) Replication-incompetent virions of Japanese encephalitis virus trigger neuronal cell death by oxidative stress in a culture system. J Gen Virol 85(Pt 2), 521-33.

Lin, Y.L., Chen, L.K., Liao, C.L., Yeh, C.T., Ma, S.H., Chen, J.L., Huang, Y.L., Chen, S.S. and Chiang, H.Y. (1998) DNA immunization with Japanese encephalitis virus nonstructural protein NS1 elicits protective immunity in mice. J Virol 72(1), 191-200.

Lin, Y.L., Huang, Y.L., Ma, S.H., Yeh, C.T., Chiou, S.Y., Chen, L.K. and Liao, C.L. (1997) Inhibition of Japanese encephalitis virus infection by nitric oxide: antiviral effect of nitric oxide on RNA virus replication. J Virol 71(7), 5227-35.

Lindenbach, B.D. and Rice, C.M. (2003) Molecular biology of flaviviruses. Adv Virus Res 59, 23-61.

Liu, T.H., Liang, L.C., Wang, C.C., Liu, H.C. and Chen, W.J. (2008) The blood-brain barrier in the cerebrum is the initial site for the Japanese encephalitis virus entering the central nervous system. J Neurovirol 14(6), 514-21.

Liu, W.J., Wang, X.J., Clark, D.C., Lobigs, M., Hall, R.A. and Khromykh, A.A. (2006a) A single amino acid substitution in the West Nile virus nonstructural protein NS2A disables its ability to inhibit alpha/beta interferon induction and attenuates virus virulence in mice. J Virol 80(5), 2396-404.

Liu, W.J., Wang, X.J., Mokhonov, V.V., Shi, P.Y., Randall, R. and Khromykh, A.A. (2005) Inhibition of interferon signaling by the New York 99 strain and Kunjin subtype of West Nile virus involves blockage of STAT1 and STAT2 activation by nonstructural proteins. J Virol 79(3), 1934-42.

Liu, X., Cao, S., Zhou, R., Xu, G., Xiao, S., Yang, Y., Sun, M., Li, Y. and Chen, H. (2006b) Inhibition of Japanese encephalitis virus NS1 protein expression in cell by small interfering RNAs. Virus Genes 33(1), 69-75.

Liu, Y., King, N., Kesson, A., Blanden, R.V. and Mullbacher, A. (1989) Flavivirus infection up-regulates the expression of class I and class II major histocompatibility antigens on and enhances T cell recognition of astrocytes in vitro. J Neuroimmunol 21(2-3), 157-68.

Luo, D., Xu, T., Hunke, C., Gruber, G., Vasudevan, S.G. and Lescar, J. (2008) Crystal structure of the NS3 protease-helicase from dengue virus. J Virol 82(1), 173-83.

Mackenzie, J.M., Khromykh, A.A., Jones, M.K. and Westaway, E.G. (1998) Subcellular localization and some biochemical properties of the flavivirus Kunjin nonstructural proteins NS2A and NS4A. Virology 245(2), 203-15.

Mackenzie, J.S., Gubler, D.J. and Petersen, L.R. (2004) Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. Nat Med 10(12 Suppl), S98-109.

Mackenzie, J.S., Johansen, C.A., Ritchie, S.A., van den Hurk, A.F. and Hall, R.A. (2002) Japanese encephalitis as an emerging virus: the emergence and spread of Japanese encephalitis virus in Australasia. Curr Top Microbiol Immunol 267, 49-73.

Mandl, C.W., Allison, S.L., Holzmann, H., Meixner, T. and Heinz, F.X. (2000) Attenuation of tick-borne encephalitis virus by structure-based site-specific mutagenesis of a putative flavivirus receptor binding site. J Virol 74(20), 9601-9.

Mani, T.R., Rao, C.V., Rajendran, R., Devaputra, M., Prasanna, Y., Hanumaiah, Gajanana, A. and Reuben, R. (1991) Surveillance for Japanese encephalitis in villages near Madurai, Tamil Nadu, India. Trans R Soc Trop Med Hyg 85(2), 287-91.

Mason, P.W. (1989) Maturation of Japanese encephalitis virus glycoproteins produced by infected mammalian and mosquito cells. Virology 169(2), 354-64.

Mason, P.W., McAda, P.C., Dalrymple, J.M., Fournier, M.J. and Mason, T.L. (1987) Expression of Japanese encephalitis virus antigens in Escherichia coli. Virology 158(2), 361-72.

Mason, P.W., Pincus, S., Fournier, M.J., Mason, T.L., Shope, R.E. and Paoletti, E. (1991) Japanese encephalitis virus-vaccinia recombinants produce particulate forms of the structural membrane proteins and induce high levels of protection against lethal JEV infection. Virology 180(1), 294-305.

McCown, J., Cochran, M., Putnak, R., Feighny, R., Burrous, J., Henchal, E. and Hoke, C. (1990) Protection of mice against lethal Japanese encephalitis with a recombinant baculovirus vaccine. Am J Trop Med Hyg 42(5), 491-9.

McMinn, P.C. (1997) The molecular basis of virulence of the encephalitogenic flaviviruses. J Gen Virol 78 (Pt 11), 2711-22.

McMinn, P.C., Marshall, I.D. and Dalgarno, L. (1995) Neurovirulence and neuroinvasiveness of Murray Valley encephalitis virus mutants selected by passage in a monkey kidney cell line. J Gen Virol 76 (Pt 4), 865-72.

Mishra, M.K. and Basu, A. (2008) Minocycline neuroprotects, reduces microglial activation, inhibits caspase 3 induction, and viral replication following Japanese encephalitis. J Neurochem 105(5), 1582-95.

Misra, U.K. and Kalita, J. (2010) Overview: Japanese encephalitis. Prog Neurobiol 91(2), 108-20.

Mitani, Y., Takaoka, A., Kim, S.H., Kato, Y., Yokochi, T., Tanaka, N. and Taniguchi, T. (2001) Cross talk of the interferon-alpha/beta signalling complex with gp130 for effective interleukin-6 signalling. Genes Cells 6(7), 631-40.

Miura, K., Onodera, T., Nishida, A., Goto, N. and Fujisaki, Y. (1990) A single gene controls resistance to Japanese encephalitis virus in mice. Arch Virol 112(3-4), 261-70.

Modis, Y., Ogata, S., Clements, D. and Harrison, S.C. (2003) A ligand-binding pocket in the dengue virus envelope glycoprotein. Proc Natl Acad Sci U S A 100(12), 6986-91.

Monath, T.P., Guirakhoo, F., Nichols, R., Yoksan, S., Schrader, R., Murphy, C., Blum, P., Woodward, S., McCarthy, K., Mathis, D., Johnson, C. and Bedford, P. (2003) Chimeric live, attenuated vaccine against Japanese encephalitis (ChimeriVax-JE): phase 2 clinical trials for safety and immunogenicity, effect of vaccine dose and schedule, and memory response to challenge with inactivated Japanese encephalitis antigen. J Infect Dis 188(8), 1213-30.

Monath, T.P., Levenbook, I., Soike, K., Zhang, Z.X., Ratterree, M., Draper, K., Barrett, A.D., Nichols, R., Weltzin, R., Arroyo, J. and Guirakhoo, F. (2000) Chimeric yellow fever virus 17D-Japanese encephalitis virus vaccine: dose-response effectiveness and extended safety testing in rhesus monkeys. J Virol 74(4), 1742-51.

Monath, T.P., McCarthy, K., Bedford, P., Johnson, C.T., Nichols, R., Yoksan, S., Marchesani, R., Knauber, M., Wells, K.H., Arroyo, J. and Guirakhoo, F. (2002) Clinical proof of principle for ChimeriVax: recombinant live, attenuated vaccines against flavivirus infections. Vaccine 20(7-8), 1004-18.

Monath, T.P., Soike, K., Levenbook, I., Zhang, Z.X., Arroyo, J., Delagrave, S., Myers, G., Barrett, A.D., Shope, R.E., Ratterree, M., Chambers, T.J. and Guirakhoo, F. (1999) Recombinant, chimaeric live, attenuated vaccine (ChimeriVax) incorporating the envelope genes of Japanese encephalitis (SA14-14-2) virus and the capsid and nonstructural genes of yellow fever (17D) virus is safe, immunogenic and protective in non-human primates. Vaccine 17(15-16), 1869-82.

Mori, R., Kimoto, K. and Takeya, K. (1970) The role of the thymus in antibody production and in resistance to Japanese encephalitis virus infection. Arch Gesamte Virusforsch 29(1), 32-8.

Mori, Y., Okabayashi, T., Yamashita, T., Zhao, Z., Wakita, T., Yasui, K., Hasebe, F., Tadano, M., Konishi, E., Moriishi, K. and Matsuura, Y. (2005) Nuclear localization of Japanese encephalitis virus core protein enhances viral replication. J Virol 79(6), 3448-58.

Mori, Y., Yamashita, T., Tanaka, Y., Tsuda, Y., Abe, T., Moriishi, K. and Matsuura, Y. (2007) Processing of capsid protein by cathepsin L plays a crucial role in replication of Japanese encephalitis virus in neural and macrophage cells. J Virol 81(16), 8477-87.

Morita, K., Tadano, M., Nakaji, S., Kosai, K., Mathenge, E.G., Pandey, B.D., Hasebe, F., Inoue, S. and Igarashi, A. (2001) Locus of a virus neutralization epitope on the Japanese encephalitis virus envelope protein determined by use of long PCR-based region-specific random mutagenesis. Virology 287(2), 417-26.

Muller, U., Steinhoff, U., Reis, L.F., Hemmi, S., Pavlovic, J., Zinkernagel, R.M. and Aguet, M. (1994) Functional role of type I and type II interferons in antiviral defense. Science 264(5167), 1918-21.

Munoz-Jordan, J.L., Laurent-Rolle, M., Ashour, J., Martinez-Sobrido, L., Ashok, M., Lipkin, W.I. and Garcia-Sastre, A. (2005) Inhibition of alpha/beta interferon signaling by the NS4B protein of flaviviruses. J Virol 79(13), 8004-13.

Munoz-Jordan, J.L., Sanchez-Burgos, G.G., Laurent-Rolle, M. and Garcia-Sastre, A. (2003) Inhibition of interferon signaling by dengue virus. Proc Natl Acad Sci U S A 100(24), 14333-8.

Murali-Krishna, K., Ravi, V. and Manjunath, R. (1996) Protection of adult but not newborn mice against lethal intracerebral challenge with Japanese encephalitis virus by adoptively transferred virus-specific cytotoxic T lymphocytes: requirement for L3T4+ T cells. J Gen Virol 77 (Pt 4), 705-14.

Nam, J.H., Wyatt, L.S., Chae, S.L., Cho, H.W., Park, Y.K. and Moss, B. (1999) Protection against lethal Japanese encephalitis virus infection of mice by immunization with the highly attenuated MVA strain of vaccinia virus expressing JEV prM and E genes. Vaccine 17(3), 261-8.

Nathanson, N. and Cole, G.A. (1970) Fatal Japanese encephalitis virus infection in immunosuppresed spider monkeys. Clin Exp Immunol 6(1), 161-6.

Nawa, M., Takasaki, T., Yamada, K., Kurane, I. and Akatsuka, T. (2003) Interference in Japanese encephalitis virus infection of Vero cells by a cationic amphiphilic drug, chlorpromazine. J Gen Virol 84(Pt 7), 1737-41.

Nazmi, A., Dutta, K. and Basu, A. (2010) Antiviral and neuroprotective role of octaguanidinium dendrimer-conjugated morpholino oligomers in Japanese encephalitis. PLoS Negl Trop Dis 4(11), e892.

Neish, A.S., Read, M.A., Thanos, D., Pine, R., Maniatis, T. and Collins, T. (1995) Endothelial interferon regulatory factor 1 cooperates with NF-kappa B as a transcriptional activator of vascular cell adhesion molecule 1. Mol Cell Biol 15(5), 2558-69.

Ni, H., Burns, N.J., Chang, G.J., Zhang, M.J., Wills, M.R., Trent, D.W., Sanders, P.G. and Barrett, A.D. (1994) Comparison of nucleotide and deduced amino acid sequence of the 5' non-coding region and structural protein genes of the wild-type Japanese encephalitis virus strain SA14 and its attenuated vaccine derivatives. J Gen Virol 75 (Pt 6), 1505-10.

Nitatpattana, N., Dubot-Peres, A., Gouilh, M.A., Souris, M., Barbazan, P., Yoksan, S., de Lamballerie, X. and Gonzalez, J.P. (2008) Change in Japanese encephalitis virus distribution, Thailand. Emerg Infect Dis 14(11), 1762-5.

Oya, A. (1988) Japanese encephalitis vaccine. Acta Paediatr Jpn 30(2), 175-84.

Pan, C.H., Chen, H.W., Huang, H.W. and Tao, M.H. (2001) Protective mechanisms induced by a Japanese encephalitis virus DNA vaccine: requirement for antibody but not CD8(+) cytotoxic T-cell responses. J Virol 75(23), 11457-63.

Paranjape, S.M. and Harris, E. (2007) Y box-binding protein-1 binds to the dengue virus 3'-untranslated region and mediates antiviral effects. J Biol Chem 282(42), 30497-508.

Pastorino, B., Nougairede, A., Wurtz, N., Gould, E. and de Lamballerie, X. (2010) Role of host cell factors in flavivirus infection: Implications for pathogenesis and development of antiviral drugs. Antiviral Res 87(3), 281-94.

Pestka, S., Krause, C.D., Sarkar, D., Walter, M.R., Shi, Y. and Fisher, P.B. (2004) Interleukin-10 and related cytokines and receptors. Annu Rev Immunol 22, 929-79.

Poland, J.D., Cropp, C.B., Craven, R.B. and Monath, T.P. (1990) Evaluation of the potency and safety of inactivated Japanese encephalitis vaccine in US inhabitants. J Infect Dis 161(5), 878-82.

Pradhan, S., Gupta, R.K., Singh, M.B. and Mathur, A. (2001) Biphasic illness pattern due to early relapse in Japanese-B virus encephalitis. J Neurol Sci 183(1), 13-8.

Qi, W.B., Hua, R.H., Yan, L.P., Tong, G.Z., Zhang, G.H., Ren, T., Wu, D.L. and Liao, M. (2008) Effective inhibition of Japanese encephalitis virus replication by small interfering RNAs targeting the NS5 gene. Virus Res 132(1-2), 145-51.

Ramakrishna, C., Ravi, V., Desai, A., Subbakrishna, D.K., Shankar, S.K. and Chandramuki, A. (2003) T helper responses to Japanese encephalitis virus infection are dependent on the route of inoculation and the strain of mouse used. J Gen Virol 84(Pt 6), 1559-67.

Raung, S.L., Chen, S.Y., Liao, S.L., Chen, J.H. and Chen, C.J. (2007) Japanese encephalitis virus infection stimulates Src tyrosine kinase in neuron/glia. Neurosci Lett 419(3), 263-8.

Ravi, V., Desai, A., Balaji, M., Apte, M.P., Lakshman, L., Subbakrishna, D.K., Sridharan, G., Dhole, T.N. and Ravikumar, B.V. (2006) Development and evaluation of a rapid IgM capture ELISA (JEV-Chex) for the diagnosis of Japanese encephalitis. J Clin Virol 35(4), 429-34.

Ravi, V., Desai, A.S., Shenoy, P.K., Satishchandra, P., Chandramuki, A. and Gourie-Devi, M. (1993) Persistence of Japanese encephalitis virus in the human nervous system. J Med Virol 40(4), 326-9.

Ravi, V., Parida, S., Desai, A., Chandramuki, A., Gourie-Devi, M. and Grau, G.E. (1997) Correlation of tumor necrosis factor levels in the serum and cerebrospinal fluid with clinical outcome in Japanese encephalitis patients. J Med Virol 51(2), 132-6.

Reuben, R. and Gajanana, A. (1997) Japanese encephalitis in India. Indian J Pediatr 64(2), 243-51.

Rey, F.A., Heinz, F.X., Mandl, C., Kunz, C. and Harrison, S.C. (1995) The envelope glycoprotein from tick-borne encephalitis virus at 2 A resolution. Nature 375(6529), 291-8.

Rice, C.M., Lenches, E.M., Eddy, S.R., Shin, S.J., Sheets, R.L. and Strauss, J.H. (1985) Nucleotide sequence of yellow fever virus: implications for flavivirus gene expression and evolution. Science 229(4715), 726-33.

Rodhain, F. (1996) [Recent data on the epidemiology of Japanese encephalitis]. Bull Acad Natl Med 180(6), 1325-37; discussion 1338-40.

Roehrig, J.T. (2003) Antigenic structure of flavivirus proteins. Adv Virus Res 59, 141-75.

Roosendaal, J., Westaway, E.G., Khromykh, A. and Mackenzie, J.M. (2006) Regulated cleavages at the West Nile virus NS4A-2K-NS4B junctions play a major role in rearranging cytoplasmic membranes and Golgi trafficking of the NS4A protein. J Virol 80(9), 4623-32.

Ruff, T.A., Eisen, D., Fuller, A. and Kass, R. (1991) Adverse reactions to Japanese encephalitis vaccine. Lancet 338(8771), 881-2.

Saini, M. and Vrati, S. (2003) A Japanese encephalitis virus peptide present on Johnson grass mosaic virus-like particles induces virus-neutralizing antibodies and protects mice against lethal challenge. J Virol 77(6), 3487-94.

Salonen, A., Ahola, T. and Kaariainen, L. (2005) Viral RNA replication in association with cellular membranes. Curr Top Microbiol Immunol 285, 139-73.

Samuel, M.A. and Diamond, M.S. (2005) Alpha/beta interferon protects against lethal West Nile virus infection by restricting cellular tropism and enhancing neuronal survival. J Virol 79(21), 13350-61.

Saxena, V., Mathur, A., Krishnani, N. and Dhole, T.N. (2008a) An insufficient anti-inflammatory cytokine response in mouse brain is associated with increased tissue pathology and viral load during Japanese encephalitis virus infection. Arch Virol 153(2), 283-92.

Saxena, V., Mathur, A., Krishnani, N. and Dhole, T.N. (2008b) Kinetics of cytokine profile during intraperitoneal inoculation of Japanese encephalitis virus in BALB/c mice model. Microbes Infect 10(10-11), 1210-7.

Scheidereit, C. (2006) IkappaB kinase complexes: gateways to NF-kappaB activation and transcription. Oncogene 25(51), 6685-705.

Scherbik, S.V., Stockman, B.M. and Brinton, M.A. (2007) Differential expression of interferon (IFN) regulatory factors and IFN-stimulated genes at early times after West Nile virus infection of mouse embryo fibroblasts. J Virol 81(21), 12005-18.

Schlesinger, J.J., Brandriss, M.W., Cropp, C.B. and Monath, T.P. (1986) Protection against yellow fever in monkeys by immunization with yellow fever virus nonstructural protein NS1. J Virol 60(3), 1153-5.

Sebastian, L., Desai, A., Madhusudana, S.N. and Ravi, V. (2009) Pentoxifylline inhibits replication of Japanese encephalitis virus: a comparative study with ribavirin. Int J Antimicrob Agents 33(2), 168-73.

Sebastian, L., Desai, A., Shampur, M.N., Perumal, Y., Sriram, D. and Vasanthapuram, R. (2008) N-methylisatin-beta-thiosemicarbazone derivative (SCH 16) is an inhibitor of Japanese encephalitis virus infection in vitro and in vivo. Virol J 5, 64.

Sebastian, L., Madhusudana, S.N., Ravi, V. and Desai, A. (2011) Mycophenolic acid inhibits replication of Japanese encephalitis virus. Chemotherapy 57(1), 56-61.

Seif, S.A., Morita, K. and Igarashi, A. (1996) A 27 amino acid coding region of JE virus E protein expressed in E. coli as fusion protein with glutathione-S-transferase elicit neutralizing antibody in mice. Virus Res 43(1), 91-6.

Seif, S.A., Morita, K., Matsuo, S., Hasebe, F. and Igarashi, A. (1995) Finer mapping of neutralizing epitope(s) on the C-terminal of Japanese encephalitis virus E-protein expressed in recombinant Escherichia coli system. Vaccine 13(16), 1515-21.

Shawar, S.M., Vyas, J.M., Rodgers, J.R. and Rich, R.R. (1994) Antigen presentation by major histocompatibility complex class I-B molecules. Annu Rev Immunol 12, 839-80.

Shen, J., SS, T.T., Schrieber, L. and King, N.J. (1997) Early E-selectin, VCAM-1, ICAM-1, and late major histocompatibility complex antigen induction on human endothelial cells by flavivirus and comodulation of adhesion molecule expression by immune cytokines. J Virol 71(12), 9323-32.

Shrivastva, A., Tripathi, N.K., Parida, M., Dash, P.K., Jana, A.M. and Lakshmana Rao, P.V. (2008) Comparison of a dipstick enzyme-linked immunosorbent assay with commercial assays for detection of Japanese encephalitis virus-specific IgM antibodies. J Postgrad Med 54(3), 181-5.

Sohn, Y.M., Park, M.S., Rho, H.O., Chandler, L.J., Shope, R.E. and Tsai, T.F. (1999) Primary and booster immune responses to SA14-14-2 Japanese encephalitis vaccine in Korean infants. Vaccine 17(18), 2259-64.

Solomon, T. (2004) Flavivirus encephalitis. N Engl J Med 351(4), 370-8.

Solomon, T. (2006) Control of Japanese encephalitis--within our grasp? N Engl J Med 355(9), 869-71.

Solomon, T., Dung, N.M., Kneen, R., Gainsborough, M., Vaughn, D.W. and Khanh, V.T. (2000) Japanese encephalitis. J Neurol Neurosurg Psychiatry 68(4), 405-15.

Solomon, T., Dung, N.M., Wills, B., Kneen, R., Gainsborough, M., Diet, T.V., Thuy, T.T., Loan, H.T., Khanh, V.C., Vaughn, D.W., White, N.J. and Farrar, J.J. (2003) Interferon alfa-2a in Japanese encephalitis: a randomised double-blind placebo-controlled trial. Lancet 361(9360), 821-6.

Solomon, T. and Winter, P.M. (2004) Neurovirulence and host factors in flavivirus encephalitis--evidence from clinical epidemiology. Arch Virol Suppl(18), 161-70.

Srivastava, A.K., Morita, K. and Igarashi, A. (1990) Immunogenicity of peptides cleaved by cyanogen bromide from Japanese encephalitis virus envelope glycoprotein E. Acta Virol 34(3), 228-38.

Srivastava, A.K., Morita, K., Matsuo, S., Tanaka, M. and Igarashi, A. (1991) Japanese encephalitis virus fusion protein with protein A expressed in Escherichia coli confers protective immunity in mice. Microbiol Immunol 35(10), 863-70.

Srivastava, A.K., Putnak, J.R., Lee, S.H., Hong, S.P., Moon, S.B., Barvir, D.A., Zhao, B., Olson, R.A., Kim, S.O., Yoo, W.D., Towle, A.C., Vaughn, D.W., Innis, B.L. and Eckels, K.H. (2001) A purified inactivated Japanese encephalitis virus vaccine made in Vero cells. Vaccine 19(31), 4557-65.

Sugawara, K., Nishiyama, K., Ishikawa, Y., Abe, M., Sonoda, K., Komatsu, K., Horikawa, Y., Takeda, K., Honda, T., Kuzuhara, S., Kino, Y., Mizokami, H., Mizuno, K., Oka, T. and Honda, K. (2002) Development of Vero cell-derived inactivated Japanese encephalitis vaccine. Biologicals 30(4), 303-14.

Sumiyoshi, H., Mori, C., Fuke, I., Morita, K., Kuhara, S., Kondou, J., Kikuchi, Y., Nagamatu, H. and Igarashi, A. (1987) Complete nucleotide sequence of the Japanese encephalitis virus genome RNA. Virology 161(2), 497-510.

Swarup, V., Ghosh, J., Duseja, R., Ghosh, S. and Basu, A. (2007a) Japanese encephalitis virus infection decrease endogenous IL-10 production: correlation with microglial activation and neuronal death. Neurosci Lett 420(2), 144-9.

Swarup, V., Ghosh, J., Ghosh, S., Saxena, A. and Basu, A. (2007b) Antiviral and anti-inflammatory effects of rosmarinic acid in an experimental murine model of Japanese encephalitis. Antimicrob Agents Chemother 51(9), 3367-70.

Swarup, V., Ghosh, J., Mishra, M.K. and Basu, A. (2008) Novel strategy for treatment of Japanese encephalitis using arctigenin, a plant lignan. J Antimicrob Chemother 61(3), 679-88.

Tadano, M., Makino, Y., Fukunaga, T., Okuno, Y. and Fukai, K. (1989) Detection of dengue 4 virus core protein in the nucleus. I. A monoclonal antibody to dengue 4 virus reacts with the antigen in the nucleus and cytoplasm. J Gen Virol 70 (Pt 6), 1409-15.

Takahashi, M. (1976) The effects of environmental and physiological conditions of Culex tritaeniorhynchus on the pattern of transmission of Japanese encephalitis virus. J Med Entomol 13(3), 275-84.

Takaku, K., Yamashita, T. and Osanai, T. (1968) Japanese encephalitis purified vaccine. Biken J. 11, 25-39

Takegami, T., Miyamoto, H., Nakamura, H. and Yasui, K. (1982) Biological activities of the structural proteins of Japanese encephalitis virus. Acta Virol 26(5), 312-20.

Tandan, J.B., Ohrr, H., Sohn, Y.M., Yoksan, S., Ji, M., Nam, C.M. and Halstead, S.B. (2007) Single dose of SA 14-14-2 vaccine provides long-term protection against Japanese encephalitis: a case-control study in Nepalese children 5 years after immunization. Vaccine 25(27), 5041-5.

Tauber, E., Kollaritsch, H., Korinek, M., Rendi-Wagner, P., Jilma, B., Firbas, C., Schranz, S., Jong, E., Klingler, A., Dewasthaly, S. and Klade, C.S. (2007) Safety and immunogenicity of a Vero-cell-derived, inactivated Japanese encephalitis vaccine: a non-inferiority, phase III, randomised controlled trial. Lancet 370(9602), 1847-53.

Thongtan, T., Cheepsunthorn, P., Chaiworakul, V., Rattanarungsan, C., Wikan, N. and Smith, D.R. (2010) Highly permissive infection of microglial cells by Japanese encephalitis virus: a possible role as a viral reservoir. Microbes Infect 12(1), 37-45.

Tsai, T.F. (2000) New initiatives for the control of Japanese encephalitis by vaccination: minutes of a WHO/CVI meeting, Bangkok, Thailand, 13-15 October 1998. Vaccine 18 Suppl 2, 1-25.

Tsai, T.F., GJ, C. and YX, Y. (1999) Japanese encephalitis vaccines, 672-710 pp. WB Saunders and Company, Philadelphia.

Tsao, C.H., Su, H.L., Lin, Y.L., Yu, H.P., Kuo, S.M., Shen, C.I., Chen, C.W. and Liao, C.L. (2008) Japanese encephalitis virus infection activates caspase-8 and -9 in a FADD-independent and mitochondrion-dependent manner. J Gen Virol 89(Pt 8), 1930-41.

Umenai, T., Krzysko, R., Bektimirov, T.A. and Assaad, F.A. (1985) Japanese encephalitis: current worldwide status. Bull World Health Organ 63(4), 625-31.

Upadhyaya, B. and Manjunath, R. (2009) Baker's yeast expressing the Japanese encephalitis virus envelope protein on its cell surface: induction of an antigen-specific but non-neutralizing antibody response. Yeast 26(7), 383-97.

van den Hurk, A.F., Ritchie, S.A. and Mackenzie, J.S. (2009) Ecology and geographical expansion of Japanese encephalitis virus. Annu Rev Entomol 54, 17-35.

Venter, M., Myers, T.G., Wilson, M.A., Kindt, T.J., Paweska, J.T., Burt, F.J., Leman, P.A. and Swanepoel, R. (2005) Gene expression in mice infected with West Nile virus strains of different neurovirulence. Virology 342(1), 119-40.

Verma, S.K., Gupta, N., Pattnaik, P., Babu, J.P., Rao, P.V. and Kumar, S. (2009) Antibodies against refolded recombinant envelope protein (domain III) of Japanese encephalitis virus inhibit the JEV infection to Porcine Stable Kidney cells. Protein Pept Lett 16(11), 1334-41.

Wang, B., Hua, R.H., Tian, Z.J., Chen, N.S., Zhao, F.R., Liu, T.Q., Wang, Y.F. and Tong, G.Z. (2009) Identification of a virus-specific and conserved B-cell epitope on NS1 protein of Japanese encephalitis virus. Virus Res 141(1), 90-5.

Wang, T., Scully, E., Yin, Z., Kim, J.H., Wang, S., Yan, J., Mamula, M., Anderson, J.F., Craft, J. and Fikrig, E. (2003) IFN-gamma-producing gamma delta T cells help control murine West Nile virus infection. J Immunol 171(5), 2524-31.

Wang, Y., Lobigs, M., Lee, E. and Mullbacher, A. (2004) Exocytosis and Fas mediated cytolytic mechanisms exert protection from West Nile virus induced encephalitis in mice. Immunol Cell Biol 82(2), 170-3.

Wathelet, M.G., Lin, C.H., Parekh, B.S., Ronco, L.V., Howley, P.M. and Maniatis, T. (1998) Virus infection induces the assembly of coordinately activated transcription factors on the IFN-beta enhancer in vivo. Mol Cell 1(4), 507-18.

Welsch, S., Miller, S., Romero-Brey, I., Merz, A., Bleck, C.K., Walther, P., Fuller, S.D., Antony, C., Krijnse-Locker, J. and Bartenschlager, R. (2009) Composition and three-dimensional architecture of the dengue virus replication and assembly sites. Cell Host Microbe 5(4), 365-75.

Westaway, E.G., Khromykh, A.A., Kenney, M.T., Mackenzie, J.M. and Jones, M.K. (1997) Proteins C and NS4B of the flavivirus Kunjin translocate independently into the nucleus. Virology 234(1), 31-41.

Winkler, G., Maxwell, S.E., Ruemmler, C. and Stollar, V. (1989) Newly synthesized dengue-2 virus nonstructural protein NS1 is a soluble protein but becomes partially hydrophobic and membrane-associated after dimerization. Virology 171(1), 302-5.

Winter, P.M., Dung, N.M., Loan, H.T., Kneen, R., Wills, B., Thu le, T., House, D., White, N.J., Farrar, J.J., Hart, C.A. and Solomon, T. (2004) Proinflammatory cytokines and chemokines in humans with Japanese encephalitis. J Infect Dis 190(9), 1618-26.

Wu, H.H., Chen, C.T., Lin, Y.L. and Lee, S.T. (2004) Sub-fragments of the envelope gene are highly protective against the Japanese encephalitis virus lethal infection in DNA priming--protein boosting immunization strategies. Vaccine 22(5-6), 793-800.

Wu, J., Bera, A.K., Kuhn, R.J. and Smith, J.L. (2005) Structure of the Flavivirus helicase: implications for catalytic activity, protein interactions, and proteolytic processing. J Virol 79(16), 10268-77.

Wu, S.F., Lee, C.J., Liao, C.L., Dwek, R.A., Zitzmann, N. and Lin, Y.L. (2002) Antiviral effects of an iminosugar derivative on flavivirus infections. J Virol 76(8), 3596-604.

Xin, Y.Y., Ming, Z.G., Peng, G.Y., Jian, A. and Min, L.H. (1988) Safety of a live-attenuated Japanese encephalitis virus vaccine (SA14-14-2) for children. Am J Trop Med Hyg 39(2), 214-17.

Yamashita, T., Unno, H., Mori, Y., Tani, H., Moriishi, K., Takamizawa, A., Agoh, M., Tsukihara, T. and Matsuura, Y. (2008) Crystal structure of the catalytic domain of Japanese encephalitis virus NS3 helicase/nucleoside triphosphatase at a resolution of 1.8 A. Virology 373(2), 426-36.

Yang, T.C., Shiu, S.L., Chuang, P.H., Lin, Y.J., Wan, L., Lan, Y.C. and Lin, C.W. (2009) Japanese encephalitis virus NS2B-NS3 protease induces caspase 3 activation and mitochondria-mediated apoptosis in human medulloblastoma cells. Virus Res 143(1), 77-85.

Yasuda, A., Kimura-Kuroda, J., Ogimoto, M., Miyamoto, M., Sata, T., Sato, T., Takamura, C., Kurata, T., Kojima, A. and Yasui, K. (1990) Induction of protective immunity in animals vaccinated with recombinant vaccinia viruses that express PreM and E glycoproteins of Japanese encephalitis virus. J Virol 64(6), 2788-95.

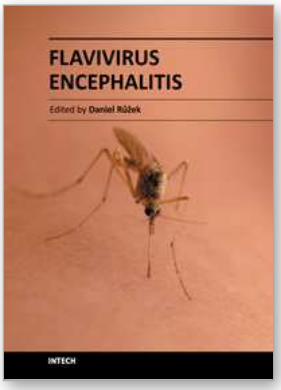
Yoo, J.S., Kim, C.M., Kim, J.H., Kim, J.Y. and Oh, J.W. (2009) Inhibition of Japanese encephalitis virus replication by peptide nucleic acids targeting cis-acting elements on the plus- and minus-strands of viral RNA. Antiviral Res 82(3), 122-33.

Yu, I.M., Zhang, W., Holdaway, H.A., Li, L., Kostyuchenko, V.A., Chipman, P.R., Kuhn, R.J., Rossmann, M.G. and Chen, J. (2008) Structure of the immature dengue virus at low pH primes proteolytic maturation. Science 319(5871), 1834-7.

Zhang, M.J., Wang, M.J., Jiang, S.Z. and Ma, W.Y. (1989) Passive protection of mice, goats, and monkeys against Japanese encephalitis with monoclonal antibodies. J Med Virol 29(2), 133-8.

Zhang, Y., Chirmule, N., Gao, G.P., Qian, R., Croyle, M., Joshi, B., Tazelaar, J. and Wilson, J.M. (2001) Acute cytokine response to systemic adenoviral vectors in mice is mediated by dendritic cells and macrophages. Mol Ther 3(5 Pt 1), 697-707.

Zhao, Z., Wakita, T. and Yasui, K. (2003) Inoculation of plasmids encoding Japanese encephalitis virus PrM-E proteins with colloidal gold elicits a protective immune response in BALB/c mice. J Virol 77(7), 4248-60.



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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