CHAPTER III
LITERATURE REVIEW

3.1 Microglia cells [12-16, 56]

Microglia are a mojor glia compenent of the CNS, play a critical role as resident immunocompetent and phagocytic cells in CNS and serve as scavenger cells in the event of infection, inflammation, ischemia and neurodegeneration. Microglia, however, are particularly sensitive to changes in their microenvironment and readily become activated in response to infection or injury. Activated microglia up-regulate a variety of surface receptors, including the major histocompatibility complex and complement receptors. They also undergo dramatic morphological changes from resting ramified cells to activated amoeboid microglia. Besides morphological changes and surface molecule upregulation, activated microglia secrete a host of soluble factors. A number of these factors, such as the glia-derived neurotrophic factor, are potentially beneficial to the survival of neurons, reminiscence of the neuroprotective role played by activated astrocytes, another major type of glial cells in the brain. Nevertheless, overactivated microglia can induce significant and highly detrimental neurotoxic effects by the excess production of cytotoxic factors such as tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β), free radicals such as nitric oxide (NO) and superoxide, fatty acid metabolites such as eicosanoids, and quinolinic acid. These stimuli can be cause of neuronal cell death and pathogenesis in neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), Amyotrophic lateral sclerosis (ALS), Huntington's disease (HD). Figure 3.1 shows mechanism of microglia activation and neurotoxicity [16].

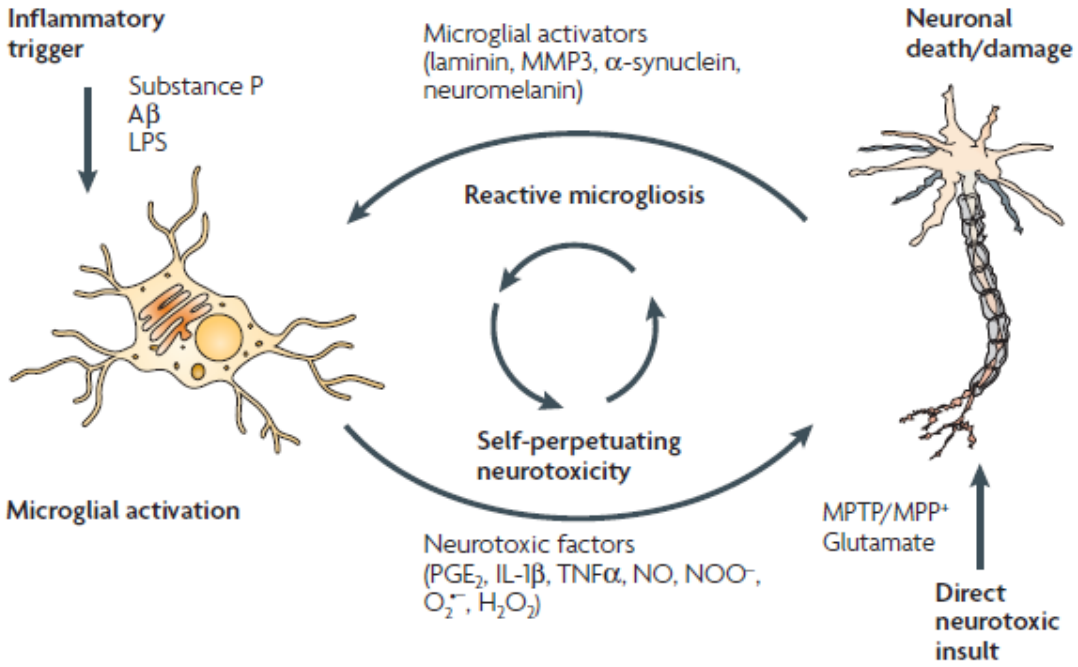

Figure 3.1 Possible mechanisms of reactive microgliosis drives progressive neurotoxicity [15]

Microglia-mediated neurotoxicity tends to be progressive, which could contribute to the progressive nature of several neurodegenerative diseases. Endogenous protective regulatory signals in the brain have been identified that inhibit microglia over activation, such as neuropeptides, cannabinoids, anti-inflammatory cytokines, oestrogen, glucocorticoids and even microglia apoptosis. It has been proposed that if they were overwhelmed by an excessive inflammatory response, microglia initiate neuronal death and drive the progressive nature of neurodegenerative disease. Table 1 summarized microglia activators that cause neurotoxicity through ROS production [15].

Table 3.1 Microglia activators that cause neurotoxicity through ROS production [15]

Microglia activator	NADPH oxidase induced ROS	Pro-inflammatory cytokine release
Rotenone	Yes	No
Paraquat	Yes	No
Substance P	Yes	IL-6
Lipopolysaccharide	Yes	No
Neuromelanin	Yes	No
α-Synuclein	Yes	No
Diesel exhaust particles	Yes	No
Gandgliosides	Yes	Yes
Thrombin	Yes	Yes / No
Amyloid-β	Yes	Yes
MMP-3	Yes	Yes

Changes in microglia phenotype relate to cellular processes including specific neurotransmitter, pattern recognition, or immune-related receptor activation. The normal functional capacity of microglia to rapidly return an injured tissue back to normal homeostasis for maintaining a healthy tissue environment. This response requires migration of microglia to the damaged area and phagocytosis of aberrant material. To accomplish this, microglia cells express many surface receptors allowing for a direct interaction with the target [16]. Microglial receptors including the presence of inwardly-rectifying potassium channels other ion channels, receptors for adenosine triphosphate, calcitonin gene-related peptide, pattern recognition receptors (PRR), and multiple neurotransmitter, neurohormone receptors and chemokines. Initiation of a microglia response can involve purinergic receptors (P2X, P2Y, the scavenger receptor CD36, toll-like receptors (TLRs), and chemokine receptors. These include the CX3C chemokine fractalkine receptor (CX3CR1) and CXCR3 that triggered by the neuronal chemokine CCL21 elevated with cell death and regulated by CXCL10. These receptors as mentioned eariler can induce changes in membrane potential, intracellular calcium, cellular motility, and cytokine release [16].

Table 3.2 Receptors and ion channels on microglia [15]

Receptor	**Expression**
TNFR1	Cell-death receptor
TNFR2	Trophic receptor / 2nd cell death receptor
IFNγR	Mi phenotype
IFNAR type 1 IFN	Suppression of glutamate and super oxide
IL-1R1 / IL-1R2	Induction of inflammatory mediators (IL-6)
IL-2Rα/β/γ(γc)	Augment NO production, M2-like phenotype
IL-10R	Induction of M2-like deactivation phenotype
IL-13R	Induction of M2-like phenotype
IL-15R	Reduced NO production, microglia survival
IL-18R	Attenuation of induced IL-12
Ion channels	**Expression**
ORAI1 / CRAC	Activation with ER Ca^{2+} depletion
TRPM7; TRPC6, TRPM2	Activation for Ca^{2+} realease/IL-6
TRPV1 ; TRPM4	Activation for Ca^{2+} release
IK1R, Kir2.1	Activated microglia
IKDR; Kv1.1; Kv1.2; Kv1.3; Kv1.5	Activated microglia
Ca^{2+}-dependent K	Increased in activated microglia
G protein-activated K^+	Metabotropic receptor activation
TTX-sensitive INA	20%rat; 95% brain tumors
Nav1.1, Nav1.5, Nav1.6	TTX blocked LPs activation
Nav1.6	Optic nerve / spinal cord
Volume-regulated Cl^-	Proliferation, phagocytosis
CLIC-1 chloride	Proliferation and ROS
Proton channels	Respiratory burst, cell volume
Aquaporins	LPS activated microglia
Connexins	Activated microglia TBI

Table 3.3 Neurotransmitter receptors in microglia [15]

Receptor	**Expression**
A1 A2A; A2B; A3	Proliferation, survival, cytokine, trophic effect
P2X1, P2X4, P2X7	Embryonic P2X1, P2X4; adult P2X4; P2X7
P2X4	Activated microglia / neuropathic pain
P2X7	Activated microglia, cytokine release
P2Y2; P2Y6; P2Y12; P2Y13	Microglia activation, migration, pain
GluR1, GluR2, GluR3, GluR4	TNF-α, cytoskeleton remodeling, chemotaxis
mGluR5a	Ca^{2+} transient
mGluR2, mGluR4	TNF-α, microglia activation
mGluR6, mGluR8	Reduce neurotoxicity
GABAB(1a); b(1b); B(1c)	Increased facial nerve axotomy microglia
α7nACh	Nicotine triggered Ca^{2+} transient
1A, α2A, β1, β2	Cytokine release
Dopamine (D1, D2)	Activated outward K^{+} currents
AMPA / KA	Only on proliferating microglia

In the case of activated microglia cells that leads to neurotoxicity, receptors which responsible in host defence and phagocytosis mechanism often mediate neuronal damage in the absence of microbial pathogen, suggesting that non-pathogenic stimuli are misinterpreted with dire neurotoxic consequences (Figure 3.2).

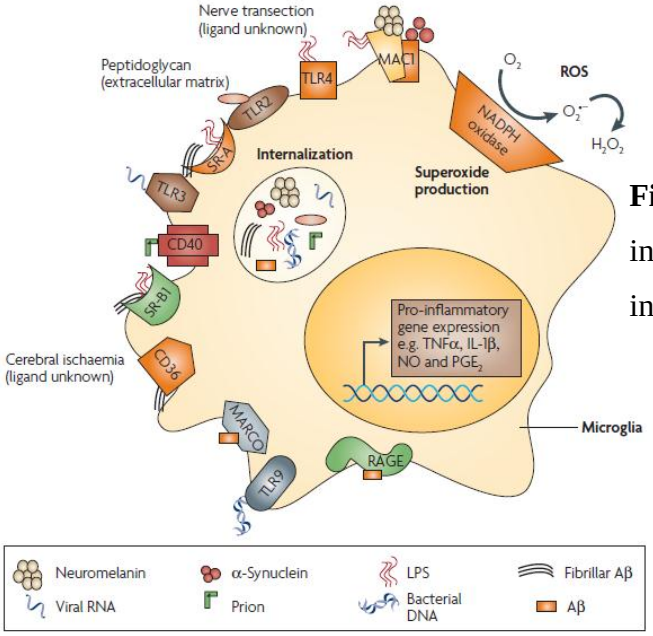

Figure 3.2 Microglia PRRs indentify neurotoxic and pro-inflammatory ligands [15]

Cells function normally with a basal level of intracellular ROS that is regulated by the antioxidant's mechanism of the cells. Upon increasing levels of intracellular ROS and depletion of the cell's antioxidative defence in response to an immunological stimulus, ROS act as 2nd messengers to amplify the pro-inflammatory function of microglia. However, higher levels of intracellular ROS might lead to microglial cell death.

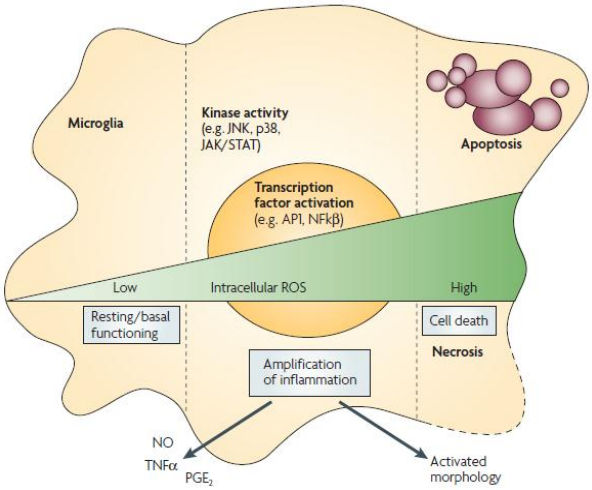

Figure 3.3 Intracellular ROS regulate microglial activation [15]

3.2 Lipopolysaccharide (LPS) [17-22]

LPS is derived from outer leaflet of the outer membrane of *Escherichia coli* [22]. Exposing HAPI cells to LPS induces the mRNAs for TNF-α and iNOS. LPS exposure also induces secretion of TNF-α and production of NO in HAPI cells. Because activation of microglia is associated with an increase in iron accumulation and ferritin expression [17]

LPS may also diffuse into the bloodstream, where it stimulates circulating immune cells. Toll-like receptor 4 (TLR4) is a pattern recognition receptor that binds to pathogen-associated molecular patterns in LPS and initiates a signaling cascade through nuclear factor κB (NF-κB)-dependent and NF-κB-independent pathways [18]. In some study have shown the evidence that PI3K dependent signaling is involved in the inflammatory responses of microglial cells following LPS stimulation, may be useful in preventing inflammatory based neurodegenerative processes [19].

The time course of LPS-induced activation of microglia is of interest. The pervious study demonstrated the release of cytokines and NO showed different time courses after stimulation by LPS. First, tumor necrosis factor alpha (TNF-α) was released with 1-hours lag time, second interleukin (IL-1β) within 3 hours and, third IL-6. Finally, NO is released in 6 hours and NO production continues for more than 48 hours. Microglia cell sthat active eith LPS have shape changes from amoeboid to a bipolar rod shape occurs for 3 to 6 hours and then returns gradually to a small round shape [20]. Before iNOS is induced, nuclear factor κB (NFκB) is activated first. It is reported that LPS induces the release of a large amount of pro-IL-1β other than mature IL-1β, although the role of pro-IL-1β is unknown [21]

Some study proposed that there has pathway involved in lipid droplet formation following microglia stimulation by LPS. The activation of TLR4 by LPS results in activation of TGFβ-activated kinase 1 (TAK1), leading to an activation of MAP kinase kinases (MKKs), and subsequent phosphorylation of JNK and p38 MAPKs. [21].

3.3 Flavivirus [23-51, 56, 69]

Japanese encephalitis virus (JEV) is an acute zoonotic infection that commonly affects children and is a major cause of acute encephalopathy. JEV is belongs to the genus *Flavivirus*, family Flaviviridae. JEV targets CNS, clinically manifesting with fever, headache, vomiting, signs of meningeal irritation, and altered consciousness leading to high mortality and neurological sequel in some of those who survive.

JEV is transmitted through a zoonotic cycle between mosquitoes, pigs and water birds. Humans get accidentally infected when bitten by an infected mosquito and are a dead end host.

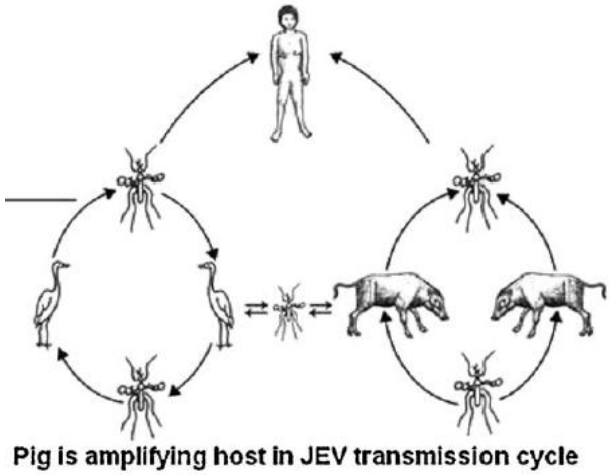

Figure 3.4 Life cycle of Japanese encephalitis virus. Human is the accidental dead end host. Pigs play a central role in the transmission JEV [25]

The JEV virion consists of a single-stranded positive sense genomic RNA, 3 structural proteins (the capsid, envelope, and membrane proteins) as well as a significant amount of lipid. The genomic RNA is 5'-capped, but lacks a 3'-polyadenylated tail and encodes for a single open reading frame flanked by noncoding 5' and 3'ends that encodes the three structural proteins (with the membrane protein being encoded as a precursor premembrane (prM) form) as well as 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).

It was proposed that macrophages can harbor JEV and the virus may employ a "Trojan horse" mechanism to infiltrate into the CNS. In this way, JEV may reside inside monocytes during transport across the BBB, leading to the subsequent infection of nearby CNS cells as the infected monocyte releases newly synthesized virus particles.

JEV infection leads to the upregulation of matrix metalloproteinase 9 (MMP-9) on endothelial cells, that causes brain damage in mice owing to the degradation of components of the basal lamina and disruption of BBB. It was also found that JEV-induced MMP-9 expression in rat brain astrocytes (one of the components of BBB, which are also infected by JEV and may help in the neuroinvasion of JEV) is mediated through a reactive oxygen species/c-Src/PDGFR/PI3 K/Akt/MAPKs dependent AP-1 signaling cascade. However, direct JEV infection of astrocytes does not result in the release of neurotoxic mediators, and it is activated microglia that promote neuronal loss. Neuronal cell death or damage can lead to the microglia activation and release of further proinflammatory mediators.

JEV-infected microglia have been shown to express elevated levels of a number pro-inflammatory mediators including IL-18, IL-1β, TNF-α, IL-6, and RANTES, which can induce neuronal cell death.

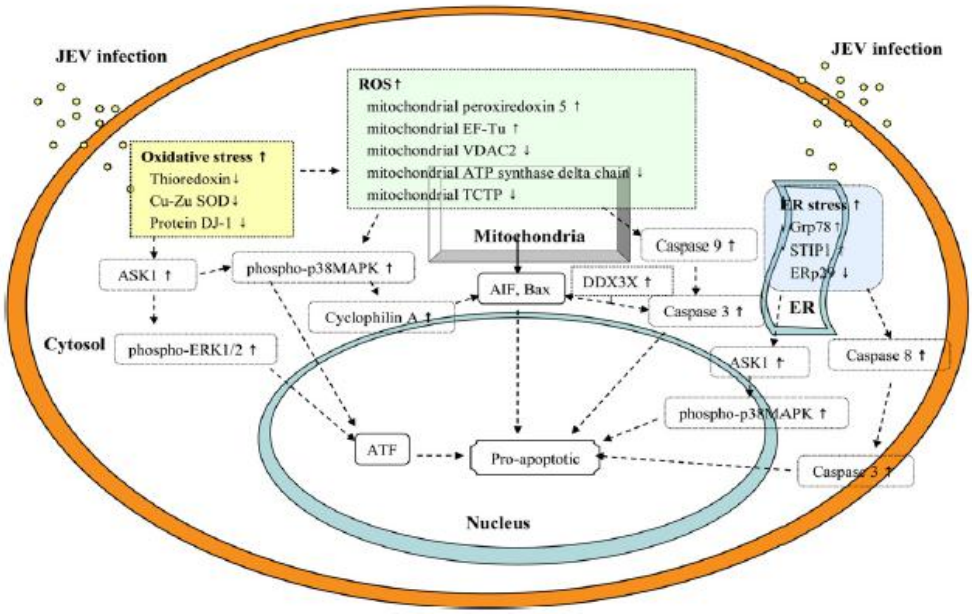

Figure 3.5 Illustrated pathway of JEV-induced apoptosis in human promonocyte cells [28]

Dengue virus

Dengue virus (DENV), of the genus *Flavivirus*, consists of a positive-sense, single-stranded RNA molecule that encodes three structural proteins (C, prM/M, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) that are involved in the assembly and replication of the virus, respectively. There are four serotypes (DENV-1 to DENV-4) that belong to the serocomplex of dengue virus. Each of these serotypes can cause disease symptoms ranging from self-limited febrile illness called dengue fever (DF) to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).

The primary targets of DENV infection are monocytes, macrophages, and dendritic cells, including the skin resident Langerhans cells.

DENV use clathrin-mediated endocytosis for entered cells and its life cycle has been illustrated below (Fig 3.6)

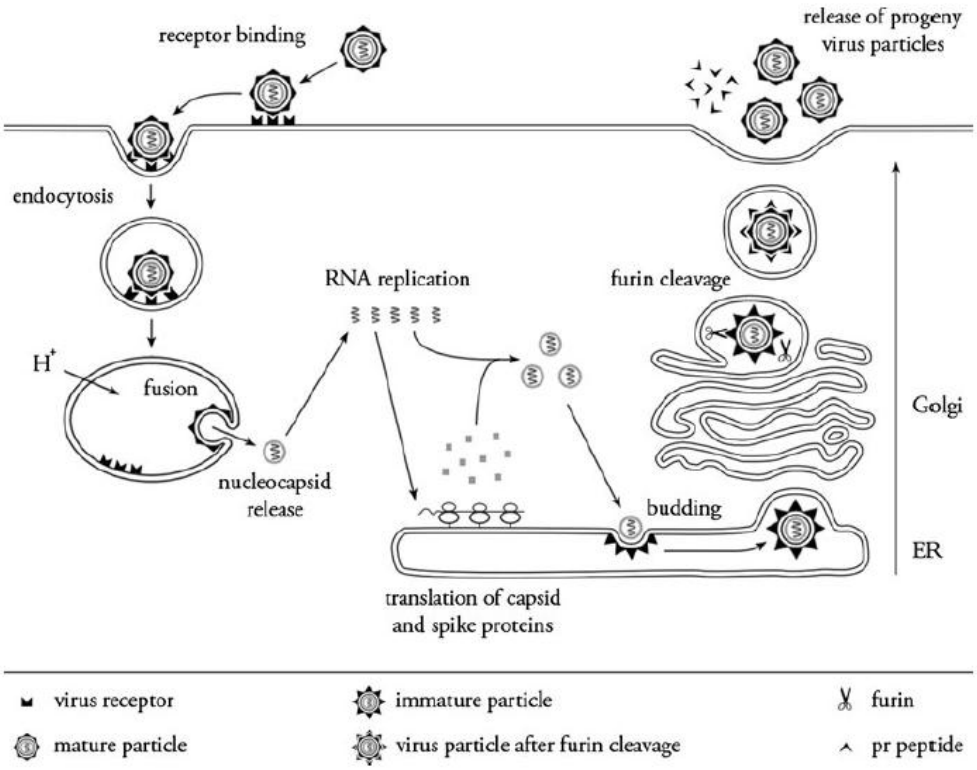

Figure 3.6 Dengue virus's life cycle [31]

Production of interferons (IFNs) is the first line defense against invading DENV. The production of IFN is initiated upon virus interaction with pathogen-recognition receptors (PRRs), e.g., C-type lectins and toll-like receptors (TLRs) that are expressed on the myeloid cells. This activates the JAK/STAT pathway. According to these responses , the cells promote the adaptive immune response through stimulation of DC maturation and by direct activation of B and T cells.

The hallmark of the pathogenesis of DHF/DSS is the loss of endothelial integrity. The level of cytokines and immunemediators has been studied such as TNF-a, IL-1b, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-18, MCP-1, and IFN-c and IFN-α are significantly increased in patients suffering from DHF/DSS.

Recent study have been proposed that antibody-mediated DENV infection of mature DCs also leads to increased levels of TNF-α and IL-6, indicating that ADE of infection may alter cytokine responses. The immonopathogenesis that caused DHF has shown below (Fig 3.7).

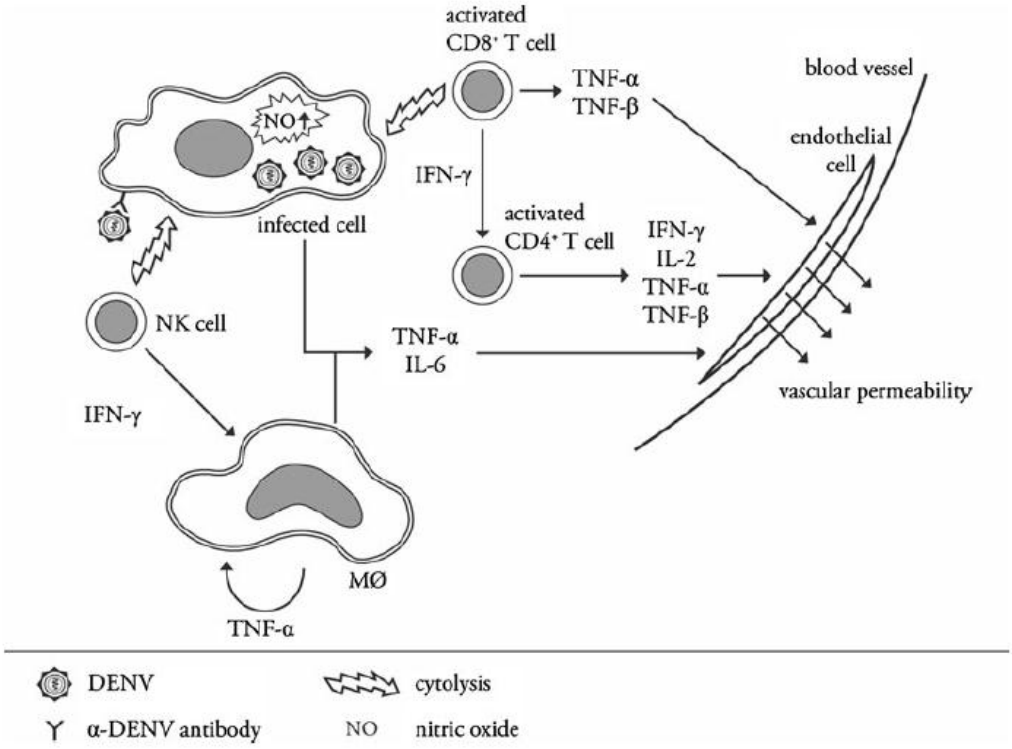

Figure 3.7 Immunopathogenesis of severe dengue-an integrated model [31]

DENV-2 16681 strains have been associated with large outbreaks of dengue fever and DHF in numerous regions of Peru since the mid-1990s, but studies to address the origins, distribution, and genetic diversity of DENV-2 strains have been limited. The DENV-2 16681 strain has been widely used in *in vivo* and *in vitro* studies involving a mouse model, a non-human primate model and permeability examination using human vascular endothelial (HUVEC) cells to elucidate DENV pathogenesis.

The DENV-2 16681 strain induced hemorrhage in mice more readily than a DENV-2 strain isolated from a DF patient, and this was attributed to its faster rate of replication and enhanced level of viraemia.

The DENV-4 strain H241 was first isolated during a dengue epidemic in the Philippines in 1956. Subsequent isolation of DENV-4 from hemorrhagic fever patients as well as patients with classical dengue fever elsewhere in Asia indicated that DENV-4 was an important pathogen.

Culex flavivirus (CxFV) is an insect-specific flavivirus globally distributed in mosquitoes of the genus *Culex* family *Flaviviridae* which includes a number of viruses of human health significance, including West Nile virus (WNV). CxFV was first isolated from *Culex pipiens* Linnaeus mosquitoes in Japan [23] but appears to have a global distribution, with additional variants identified in other *Culex* species and subspecies from Mexico, Uganda, Trinidad, and the United States.