Chapter

Pathogenesis and Host Immune Response during Japanese Encephalitis Virus Infection

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Abstract

Japanese Encephalitis Virus (JEV) is a mosquito borne flavivirus infection. Transmission of JEV starts with the infected mosquito bite where human dermis layer act as the primary site of infection. Once JEV makes its entry into blood, it infects monocytes wherein the viral replication peaks up without any cell death and results in production of TNF-α. One of the most characteristics pathogenesis of JEV is the breaching of blood brain barrier (BBB). JEV propagation occurs in neurons that results in neuronal cell death as well as dissemination of virus into astrocytes and microglia leading to overexpression of proinflammatory cytokines. JEV infection results in host cells mediated secretion of various types of cytokines including type-1 IFN along with TNF-α and IFN-γ. Molecule like nitrous oxide (NO) exhibits antiviral activities against JEV infection and helps in inhibiting the viral replication by blocking protein synthesis and viral RNA and also in virus infected cells clearance. In addition, the antibody can also acts an opsonizing agent in order to facilitate the phagocytosis of viral particles, which is mediated by Fc or C3 receptor. This chapter focuses on the crucial mechanism of JEV induced pathogenesis including neuropathogenesis viral clearance mechanisms and immune escape strategies.

Keywords: Japanese encephalitis virus, Neuropathogenesis, Dendritic cells, Macrophages, Dendritic cells

1. Introduction

Japanese Encephalitis Virus (JEV) infection is a mosquito-borne zoonotic infection in human which is the most common cause of viral encephalitis in Southeast Asia [1]. The first case of JEV was reported in Japan in the year of 1871. The virus was first isolated in the year 1935 from human brain, which was a fatal case. JEV is responsible for causing a high morbidity and high mortality specifically in the pediatrics age group [2]. Transmission cycle of JEV includes pigs which act as the reservoir/amplifying-host, water bird as carriers and mosquitoes as vector and humans are considered as the dead-end host. JEV is transmitted into human via infected Culex mosquitoes bite and thereby infected individuals develop viremia [3]. Transmission cycle starts mostly post-monsoon where the chance of mosquito breeding increases in paddy fields. JEV is single stranded positive- sense envelope RNA virus and is a member of family *Flaviviridae*. The genome of JEV encodes a

one single open reading frame (ORF) encoding single polyprotein that gets cleaved into three structural proteins namely as Capsid (C), Envelope (E), precursor to membrane protein (prM) and seven non-structural proteins including NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5 [4]. Based on the genomic variability, JEV has been categorized into five genotypes. The most prevalent genotypes seen in the northern region are I and III and that in southern region are II and IV and also, a putative genotype V [5]. The incubation period of JEV infection is 5–15 days and symptoms includes from febrile illness to a severe disease with patients showing meningoencephalitis, aseptic meningitis or a polio-like acute flaccid paralysis [6].

2. Immune cell targets employed by JEV in peripheral and central nervous system

Transmission of JEV starts with the infected mosquito bite where human dermis layer act as the primary site of infection. JEV replication occurs in peripheral system including PBMCs wherein the macrophages, dendritic cells (DCs) and monocytes become infected [7]. Such infection in peripheral system gets cleared off due activation of immune system, and that is the reason for low level of viremia in the blood [8]. During any viral infection, antigen presenting cells (APCs) such as dendritic cells (DCs) and macrophages are the first cell types that trigger the cellular immune responses. They produce various cytokines, which includes IL-6 and TNF-α, and many other pro-inflammatory cytokines. Once JEV makes its entry into blood, it infects monocytes wherein the viral replication peaks up without any cell death and results in production of TNF-α [9] that results in the activation and differentiation of monocytes into monocyte-derived dendritic cells (MDDCs) and monocyte-derived macrophages (MDMs). JEV has developed various immune escape strategies. JEV impairs with the process of DC maturation and where immature human monocyte-derived DCs (im-MDDC) helps in viral replication which takes place by surface expression of co-stimulatory cytokines/chemokine surface receptors [10]. JEV replication has been shown to take place in DCs via reducing the expression of co-stimulatory cytokines, hindering the T-cell activation and by escalating the Treg cells differentiation [11].

During JEV infection, interaction of host-pathogen in the monocyte cell lineage such as monocyte-derived macrophages (MDMs) increases the severity of the disease [12]. Macrophages acts as a hub of viral replication but in case of JEV infection, the productive replication of virus is limited followed by the increase sensitivity to the IFN response [13]. JEV modulates macrophages and DCs in distinguishing pattern. Macrophages get modulated through classical pathway by up regulating co-stimulatory molecules. DCs infected by JEV produce one of the anti-inflammatory cytokine, IL-10 and some of the pro-inflammatory cytokines such as TNF-α, IL-12 and IL-6, whereas macrophages infected by JEV does not produces IL-10 [14]. Such modulation of DCs and macrophages induces an inflammatory environment which then helps in permeability of BBB (blood brain barrier) and hence, the virus tends to spread into central nervous system (CNS). Infection of CNS causes functional damage to DCs including splenic DCs. Since dendritic cells helps in activating naïve T cells, their damage leads to an increase in viral circulation in CNS and hence, reduces the $CD4^+$ and $CD8^+$ T-cells response [15]. Although the mechanism of viral entry into the brain is not well understood but once it enters the brain cells, JEV is detected in cerebrospinal fluid (CSF) and in the nervous tissue [16]. One of the most characteristics pathogenesis of JEV is the breaching of BBB [17]. Neuron being the most important target cell during JEV however, when the infection gets into CNS, along with the neuronal cells, astrocytes also gets infected,

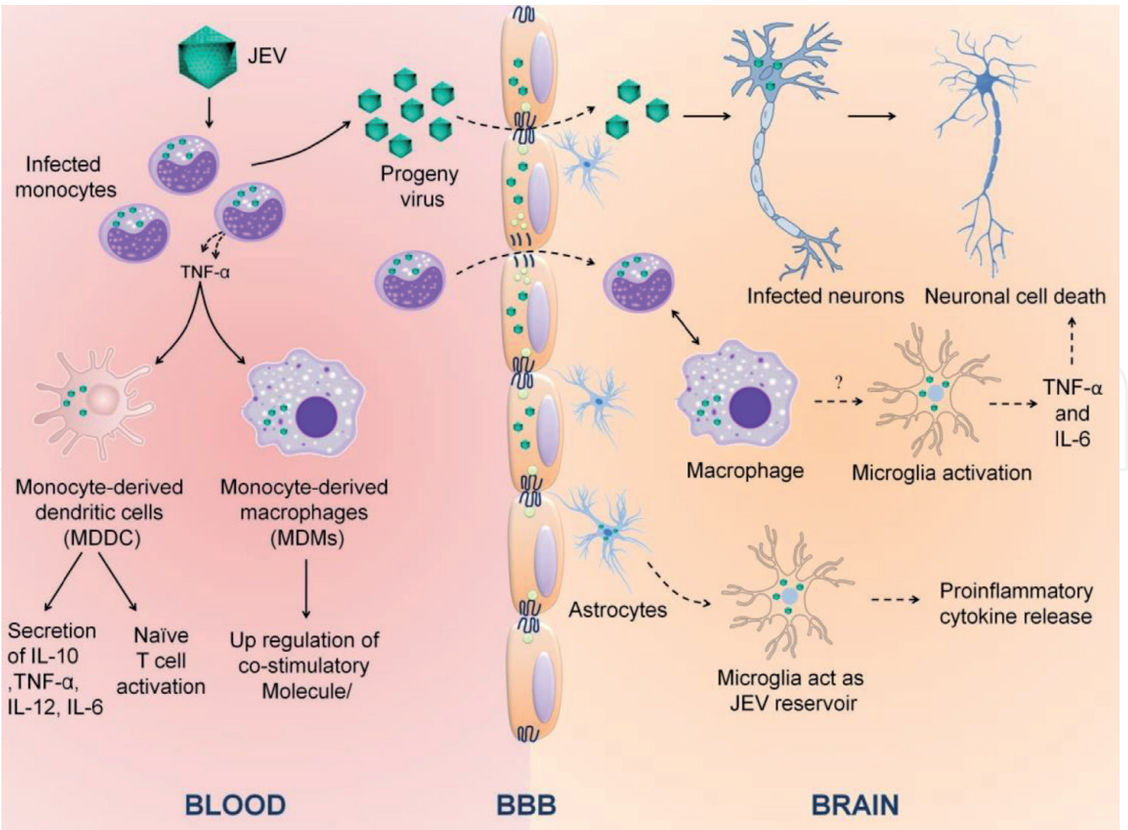

Figure 1.
Mechanism of Japanese encephalitis virus infection and involvement of immune cells. In blood JEV primarily infects monocytes and results in TNF-α production that results in the activation and differentiation of monocytes into monocyte-derived dendritic cells (MDDCs) and monocyte-derived macrophages (MDMs). These cells are involved in naïve T cell activation, secretion of TNF-α and IL-6. JEV can cross the blood brain barrier (BBB) via direct or transmigration of virus harboring monocytes. After entry into the brain, JEV can infect neuronal cells that results in cell death. The transmigrated monocytes differentiate into macrophages which may disseminate the virus to microglia which act as the viral reservoir. Astrocytes are known to disseminate the virus to microglia which results in the activation of microglia leading to over expression of proinflammatory cytokine release.

which is a constituent of BBB and an important part of CNS. Astrocytes are also considered to be helping in the transmission of JEV to the cerebrospinal fluid from peripheral tissues. The microglial cell that is considered to be the resident immune cells/macrophage of CNS is also infected by JEV. Microglial cells play a very significant role in CNS during the JEV infection via acting as a virus reservoir [18]. Upon activation microglia produces proinflammatory cytokines like TNF-alpha and IL-6, which induce death of neuronal cell (**Figure 1**) [19].

3. Neuropathogenesis during JEV infection

The pathogenesis of JEV needs to be explored at dual phases in human which initiates at the peripheral tissues and then, involvement of central nervous system (CNS). Before entering into CNS, JEV replicates in the langerhans cells (skin dendritic cells), which gets transported into the lymphatic and peripheral tissues which results in increased viremia. During the initial infection in periphery tissue, the $CD8^+$ T cell response prevents the dissemination of the JEV into the CNS. Lymphocytes harboring JEV can cross the BBB and via endocytosis process to penetrate the endothelial surface of CNS [20]. However, inability of host to produce antibodies against the infection and the immune evasion strategies of the virus makes this infection lethal. JEV propagation occurs in neurons that results

in neuronal cell death. Neuronal cell death occurs via two mechanism; direct and indirect neuronal killing. Direct killing involves the JEV propagation inside the neuronal cells that results in cell death and indirect killing involves aggressive and intense inflammatory responses leading to up-regulation of inflammatory cytokines and reactive oxygen species that causes death of neurons [21]. In addition to cell death, proliferation and growth of neuronal progenitor cells (NPCs) also gets affected which could be the possible reason for the destructive neurological cases in JE survivors [22]. JEV can also cause abnormal neuronal development in fetus via crossing transplacental barrier [23]. In order to prevent the JEV pathogenesis, virus clearance from the peripheral nervous tissues during the initial phase of infection is crucial for designing effective therapy. Clearance of virus-infected cells and recovery during JEV infection relies on the several factors including IgM antibodies, T-lymphocytes and CXCL10 mediated viral clearance by neuronal cells [24].

4. Clearance of JEV by diverse immune cell types

During JEV infection, viral clearance via immune cells is a multiple step process which involves both innate and adaptive immunity. The initial step focuses on the inhibition or on limiting the spread of virus to any new cells. In addition, already infected cells are then either eliminated or replication of JEV is suppressed permanently. However, mechanism of virus clearance during JEV infection in the CNS tissue requires immense understanding of the level of JEV infection in the CNS tissue. One of the most reliable methods is cytolysis, either immune cytolysis or virus-induced. This method involves complete removal or elimination of virus infected cells or cells where the virus is propagating. The immunological processes that are required for clearance of virus are cell-type specific. However, in case JEV infection, the virus invade the host immune cells by cytolytic mechanism and hence, inhibiting the progression of NCP (neural progenitor cells pool). In order to combat such invasion, the activation of brain macrophages is crucial which gets initiated with the help of nerve cells. These macrophages then mediate non-cytolytic viral clearance by producing IFN-β and by supporting production of T-cells that eventually produces IFN-γ [25]. Further, virus secretes proteins/factors and makes cytokine imbalance and suppresses MHC-I present on the membrane surface.

In response to the JEV infection, several mechanisms of innate immune response get activated. After getting infection, host cells starts producing various types of cytokines including type-1 IFN along with TNF-α and IFN-γ. These cytokines induces inflammatory responses and hence, inhibits the viral replication. Furthermore, the IFN-α and IFN-β binds to the NK cells and initiates the lytic activity and hence, kills the JEV infected cells. This antiviral activity gets initiated by one of the cytokine IL-12 which produced at an early phase of infection. IFN-γ then activates the brain macrophages that expresses MHC-II molecules and subsequently, helps in more cytokine production that result in inhibition of viral replication [26]. Other than the cytokines, molecule like nitrous oxide (NO) also evidently exhibits antiviral activities against JEV infection and helps in inhibiting the viral replication by blocking protein synthesis and viral RNA and also in virus infected cells clearance [27]. Adaptive immune response is highly specific involving antigenic specificity display, self/non-self recognition, and immunologic memory. Generally, during flavivirus infection, the antibodies produced by the host cells along with the complement proteins helps in the destruction of the viral particles [28]. However, in case of JEV infection, the virus tends to evade and slips through the complement mediated mechanism of host cells and by inhibition of classical pathway. Additionally, the receptor present on the macrophages interacts

with the components of the viral antigens and helps in generating soluble proteins, which then triggers adaptive immune responses promoting clearance of virus infected cells.

5. Cell-mediated immune mechanisms for JEV Clearance

In the process of clearance or elimination of JEV infected cells, cell mediated immunity plays a vital role. Cytokines playing the lead in this mechanism is IFN-γ and IL-2 secreted by T-helper (Th) cells or T-cytotoxic (TC) cells. IL-2 helps in the alteration of naïve T cells into virus-specific cytotoxic T lymphocytes (CTL) generation, which then eventually causes killing of virus infected cells [29]. However, in case of JEV or any flaviviral infection, post exposure to the infected cells, the virus controls the release of CTL and other cytokines. The stimulated Th cells generates cytokines which includes IFN-γ, IL-2, IL-6 and TNF-α which tends to disturbs the cellular activities of JEV and hence, protecting the host from viral infection. These effector molecules are produced by TH1 cells, $CD4^+$ and $CD8^+$ Tc cells, which mediate anti-viral response in order to initiate cell-mediated immune responses. NO amongst these cytokines, both IFN-γ and TNF-α could possibly help in peripheral virus clearance but not from the CNS. IFN-γ helps in maintaining anti viral properties in host cells and IL-2 then, converts naive T cell (CTL) into effector T cell and hence, activates NK cells which in return eliminates virus infected cells or virions.

6. Humoral immune mechanism for JEV clearance

During JEV infection, the host cell recruits humoral immune mechanism, which is a very significant mechanism in the process of protection against the infection in human. Once the host cells get infected by the virus, host humoral responses initiates the process by identification of virus with the recruitment of Th cells that responds to the viral antigens. These Th cells then present these viral antigens or proteins to the B cells along with the help of macrophages. Subsequently, the B cells loaded with viral antigen then get converted to plasma cells and post expansion, starts secreting Abs after few days post JE infection. Thus, this is the initiation of the humoral immune response mediated by antibody production [30]. The antibody binds to the epitopes necessary for the fusion of viral envelope with the plasma membrane and thus, blocking the penetration of virus molecules into the host cells. Furthermore, the antibody can also acts an opsonizing agent in order to facilitate the phagocytosis of viral particles, which is mediated by Fc or C3 receptor. Thus, the mechanism of inhibition of virus propagation and reduction of virus generated cytopathic effects is shown by the JE infected neutralizing antibodies. Host immune responses are triggered 4–7 days post infection resulted after structural and non-structural proteins of virus and host cells interaction.

7. Complement system against JEV infection

Viruses are a kind of pathogen that depends completely upon the host for its survival and its replication. Hence, in order to survive the virus has developed immune escape mechanism from complement system by secreting many inhibitory proteins/cytokines. However, the complement system has an adversary part to play in cases of any *Flavivirus* infection including JE due to it's of multi-component system. This adverse action takes place by either restricting the viral propagation

and hence, protecting the host or by triggering an intense inflammatory response, which increases the disease severity. The complement system helps in destruction of the viral particles by complement-dependent lysis of virus, by opsonization, by modulating functions of B-cell and T-cell and also, by phagocytosis [31]. The other pathways in which the viral particles or antigens gets destructed by the complement system is the antibody-dependent and antibody-independent pathways. This action of viral recognition and then its clearance by complement system is carried out by a group of serum proteins along with molecules presented on cell surface. Most of the proteins are presented in an inactive form which then gets activated by three main patterns through which complement system gets activated. These are the classical pathway, alternative pathway and lectin pathway. All the three pathways get started when C3, a proteolytic fragment promotes the cell uptake, which initiates the complement system. The lectin and the alternative pathway get activated by the binding of the mannose-binding lectin (MBL) present on the surface of cell and by the hydrolysis of C3, respectively. These complement system is comprised of four kinds of serum proteins; C3, factor D, factor b and properdin. A more stabilized complex C3b Bb is formed when C3b binds to B (serum protein) with the help of Mg + ions. Also, the unhydrolyzed C3 can possibly produce more of C3b, which gets deposited on the cell surface. Further, C3b Bb 3b complex known to show C5 convertase activity, and eventually, C3b-C5 and 5b component is formed commencing the first step of viral lysis [32]. This complement-mediated pathway to inhibit the contact target chosen by JEV or any flavivirus may help to understand the possible way of combating the infection [33]. The alternative pathway amplifies the activation triggered by the former two pathways and hence, heavily destroys the virus particles. The classical pathway is an antibody dependent process wherein the antibody stimulates the phagocytic cells. The initiation step is the binding of C1q (a polyvalent molecule) with the antigen–antibody complexes, which is present on the surface of a pathogen or by binding directly to the viral protein. Further, C1 complex binds to the cleaved molecules of C4 (C4a and C4b) and furthermore, C2 can complex with C4b and form a new product C4b2b that shows efficient C3 convertase activity, which is essentially required to activate C3 protein. Finally, along with C3, taking into account, C3b and C4b2b with C5 enzyme, a very efficient complex is formed for the destruction of viral particles. Thus, all three pathways converse in a common sequence of events and cause heavy cell lysis of pathogen.

8. Conclusions

Flaviviruses like JEV has taken the infection strategy into an advanced level by evading the detection machinery of the host-immune mechanism which is in responses to any viral infection to kill the virus- infected cells. This is the point where it has become a necessity of the moment to carry extensive studies on this subject. JEV modulates the host machinery in dual ways that is, by virus-mediated damage and by host- immune responses. JEV alters or inhibit both the innate and adaptive immune responses of the host. Since, no viral antigen is presented by the macrophages which are infected during the early infection phase, no adaptive immune responses is triggered during this phase. Escaping all the immune responses, JEV manages to disseminate into the CNS and causing damage to the CNS is what makes JEV infection more lethal. Any renewal and replenishment of tissues in CNS after infection is challenging. Host responses like CTL activation has been efficient in combating the virus induced MHC molecules. However, JEV comes with a counter viral strategy by activating the non-classical MHC molecules, since, these non -classical MHC molecules inhibits NK cells by binding to its receptors.

NK cells are the crucial cells so as to say, which are efficient to kill the virus infected cells. To get a clear understanding of how to combat the viral immune escape strategy, many viral antigens/proteins are profiled and characterized. Such studies are a new approach towards generating effective vaccines against JEV along with other flaviviruses. Because of all these challenging viral strategies, it has become a necessary research step to reach to a point where viral eradication shall be feasible. In order to reach to this point, basic understanding of the JEV strategy on how JEV manages to trigger imbalance between host's immunopathological and shielding mechanisms is very important. To combat spread of such lethal infection, vaccination against it should be made mandatory in the entire endemic region. Effective surveillance in the endemic region has to be considered especially in pediatric age, since; this age group is the mostly effected and has proved to be lethal.

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Conflict of interest

The authors declare no conflict of interest.

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