CHAPTER II: REVIEW OF LITERATURE

2.1 Immune response to Dengue

In response to a primary DENV infection, the immune system largely responds as expected, with an IgM response to dengue antigens occurring first and an IgG response (comprising mostly of IgG1 and IgG3 subtypes) subsequently. There is a decreased IgM response and an increased IgG response during a subsequent infection[1]. The fact that an immune response to both homotypic and heterotypic DENV is observed after either a first or second DENV infection is what makes dengue infection so fascinating. The adaptive immune response that arises after contracting any DENV offers permanent protection against the homologous virus, but it is transient against heterologous DENVs. All the DENV serotypes share a sizeable proportion of their structural antigens and this causes type-specific and cross-reactive antibodies to be produced after infection with one DENV. Studies have shown that individuals with having dengue history are at higher risk of severe dengue. Immune response to DENV protection is controversial as a sufficient number of antibodies neutralize infection when bound to virus particles but when the concentration is below the threshold, neutralizing antibodies promote disease severity[2]. Higher titers of neutralizing antibody have been linked to a decreased risk of symptomatic infection in children in a longitudinal cohort study conducted in Nicaragua[3]. It is demonstrated that primary infection is predominantly type-specific, whereas following secondary infection, cross-reactive neutralizing antibodies against heterologous DENV is developed. Upon secondary infection, the dengue virus uses this cross-reactive neutralizing antibody to enter the host cell through the Fc receptor. The term Antibody-Dependent Enhancement (ADE) is used to describe this phenomenon. ADE plays a crucial role in invading host immune cells and enhancement of disease [4]. One possible reason for ADE's variable degree of response in patients is that it is exclusively mediated by various types or specificities of antibodies. Afucosylated IgG1s, for example, have a higher binding affinity to FcγRIIIa anb FcγRIIIa; this may lead to a greater absorption of DENV immune complexes and widespread inflammation, both of which are frequently linked to ADE. Dengue patients' fucosylated IgG1s identify the E proteins, and higher antibody levels were associated with a worsening of the illness. Additionally, in the early stages of the disease, secondary DENV infection was found to have higher afucosylated IgG1 abundance than original infection, and these antibodies were indicative of severe dengue[5, 6].

2.2 Dengue Virus Cellular Receptors and Tropism

DENV is tropic and known to primarily infect human dendritic cells (DC), monocytes, and macrophages, and which are known to express cytokines. DENV initially infects the dermal and epidermal resident cells shortly after the insect bite. Among the skin cells known to be involved in DENV tropism are dermal macrophages, Langerhans cells (LC) , dermal DCs (CD1c+ and CD14+), blood-derived monocytes, keratinocytes, fibroblast, endothelium, and mast cells.[7-10]. Two significant things happen when these cells get infected. DENV replicates robustly in secondary lymphoid tissue, where it is first activated and migrates via the lymphatic system. Secondly, it recruits immune-competent cells through the release of cytokines and chemokines. In both human and mouse models, monocytes, macrophages and DC are the main targets of viral infection. The DENV infection cycle starts when the virus attaches itself to the target cell by interacting with cell surface attachment/receptor molecules and viral surface proteins. Entry of the virus particle is made possible by this contact, which often involves receptor-mediated endocytosis. Following the internalization of the virus, the union of the viral envelope with the endosomal membrane allows the virus's genome to reach the cytoplasm[11].

The interaction between components of the cellular plasma membrane and virus surface proteins is what allows target cells to recognize viruses (Fig. 1). The location and the amount of cell receptors determine how susceptible the host tissues are to the virus, making these receptors important targets for the creation of antiviral medications. First contact with the virus is usually caused by attachment factors that are deposited on the cell surface. Through non-specific binding, the virus is concentrated on the cell surface and is made easier to attach to its specific receptor that will facilitate the virus's entry into the target cell. In mammalian and mosquito cells, several candidates of different nature have been identified. These include glycosaminoglycans, which include lectins and heparan sulphate; adhesion molecule of dendritic cells (DC-SIGN), macrophages' mannose receptor (MR), and stress-induced proteins, which include ER chaperonin GRP78 and heat-shock proteins 70 and 90. This shows that instead of using a single, particular receptor to enter a cell, DENV identifies and binds to a variety of molecules, possibly in a way that is specific to a serotype. This is consistent with the wide range of cell types that can become infected, found in both mosquito and vertebrate hosts. Heparan sulphate was the first molecule identified to be involved in DENV entry. Highly sulfated

Glycosaminoglycans (GAGs) are widely distributed molecules found on the surface of several cell types. They also serve as a mediator for viral attachment. Electrostatic interactions have been seen between the negatively charged carbohydrate moieties found in GAGs and the E glycoprotein of the dengue virus. DC-SIGN is the most significant attachment factor for DENV entry that has been identified thus far[12, 13]. In fact, the search for the DENV receptor raises the possibility that the virus evolved to be non-specific by expanding its range of target molecule/ receptor for host invasion. This reflects the extensive infection of the virus within the host organism in vivo, which results in the variety of clinical presentations observed in people infected with DENV.

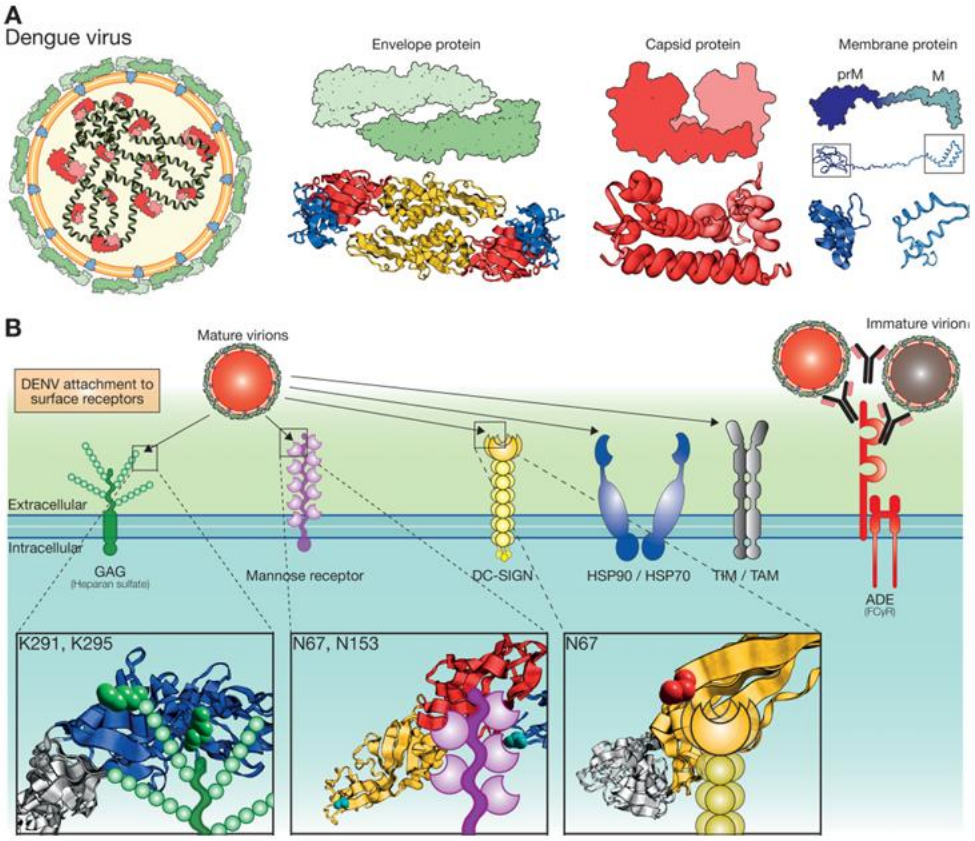

Figure 2.1. Structure of Dengue virus and the receptors. (A) Schematic representation of DENV structure .envelope protein homodimers and the membrane proteins, capsid protein homodimers (B) Cell surface receptors involved in DENV entry: mannose receptor, GAGs, , DC-SIGN, HSP90/HSP70 and TIM/TAM, and Fcγ receptors. The image was adapted from a review article published previously[11].

2.3 Cytokines in Dengue

Cytotoxic T lymphocytes (CD8+) are in charge of lysing infected cells during the DENV

immune cellular response. This process is mediated by granzymes and perforin. One of dengue's primary targets, APC cells, expose naive CD8+ T lymphocytes to viral antigens once they are inside lymphoid organs, which causes the cells to activate, proliferate, and migrate towards the infection site. The viral antigens attached to HLA class I are then presented by these effector CD8+ T lymphocytes, enabling them to identify DENV-infected cells. T-cells recognise target cell peptide-MHC complexes and release a wide variety of cytokines in response [14]. Th2 type cytokines, such IL 4, are less commonly produced by T cells than IFN-γ, TNF, CCL4, IL2[15]. An excessive or skewed profile of cytokine expression which is generally known as "**cytokine storm**" is observed in DENV infection. It is a result of an excessive immune activation that sets off a chain reaction of cytokine production. This phenomenon causes leakage and increasing capillary permeability thereby affecting the vascular endothelial cells. Using sera from DHF/DSS patients, the cytokine storm hypothesis has also been investigated in many countries where higher levels of TNF-a, IL-10, and IFN-γ have been noted. In severe dengue, elevated levels of IL-10 is prominent. However, IL-6 and chemokines like IL-8, CCL2/MCP-1, and CXCL10 also contribute to disease [16]. Given below are list of cytokines we have analysed in our study.

IL12p70

Interleukin (IL)-12 is a potent inducer of cytokine production for preactivated T and NK cells. It enhances the cytotoxic activity of cytotoxic T cells and NK cells, and stimulates T or NK cells to produce interferon (IFN)-γ. In a study conducted compared to DHF patients infected with DENV1, those with DHF who were infected with DENV2 had noticeably greater levels of IL-12p70[17].

TNF alpha

Tumor Necrosis Factor-alpha (TNF-α) is produced by monocyte at early infection stage. This proinflammatory cytokine TNF-α has an antiviral function and is directly linked to cell death. It may also be associated with the severity of the disease in DENV infections. It is well recognized that TNFα increases vascular permeability and initiates apoptotic cell death[18]. Research has indicated that elevated TNFα levels are linked to increased vascular permeability in Dengue patients.

IL10

Interleukin 10 (IL-10) is produced by monocytes in response to DENV infection. It is commonly seen to be elevated in severe dengue patients' plasma. [19]. Elevated IL-10 levels have previously been suggested as a measure of dengue fever severity[20]. A connection between plasma leakage and dengue's IL-10 levels was shown by Libraty et al[21]. A positive correlation between elevated levels of IL-10 and viral NS1 proteins in acute dengue infection patients' serum is also reported.

IL6

Interleukin-6 (IL6) is a cytokine that has been reported to maintain body temperature during acute cold exposure and is crucial for triggering the fever response during infection. The production of anti-platelet and anti-endothelial autoantibodies, coagulation abnormalities resulting in bleeding, and plasma leakage in dengue infections are all possible outcomes of interleukin-6 (IL-6)[22, 23]. It controls neutrophil activation, degranulation, and selective chemotaxis. Expression of mRNA encoding for IL-6 and other cytokines was higher in newborn mice with experimentally generated dengue encephalitis, indicating a potential link between dengue encephalitis and IL-6 production.

IL1β

Proinflammatory cytokine IL-1β is generated during inflammation after activation of the inflammasome. Clinically, dengue patients also have higher levels of IL-1β, suggesting that inflammasome activation plays a part in human DENV infections[24]. DENV induces vascular leakage and tissue injury by promoting the activation of interleukin (IL)-1β.

2.4 Chemokines in Dengue

Chemokines are a subclass of cytokines known that recruit and induce the chemotactic migration of other cells to a specific region to affect a range of biological processes, such as inflammation and homeostasis[25]. Given below are some chemokines we have used to analysed in Dengue patient’s sera in our study.

CXCL10

CXCL-10 is a chemokine, that plays an important role in the innate immune response and is also responsible for inducing IFN- -γ. CXCL10 attaches itself to the lymphocyte-expressed CXCR3 receptor. Activated T and NK cells are drawn to the infection site by CXCL10 in DENV infections.

CCL2

Monocyte Chemotactic Protein-1 (MCP-1), or CCL2, is a CC-chemokine that recruits memory T cells and monocytes to the locations where it is secreted. During DENV infection significant increase in CCL2 was observed in patient sera[26].

CXCL9

CXCL9 is also known as monokine induced by interferon-γ (MIG). It uses CXCR3 receptor to mediate its biological function. T cells with high levels of CXCR3 expression, mainly of the Th1 phenotype, are recruited by CXCL9. This cytokine is secreted from leukocytes, neutrophils, eosinophils monocytes and endothelial cells in response to infection. Expression of CXCL9 is upregulated during DENV infection ans a study also reported its association with the severity of the disease[27].

IL8

Interleukin -8 (IL8) is released by T lymphocytes, fibroblasts, eosinophils, mononuclear macrophages, and epithelial cells. It directs the neutrophils to the infection site, acting as a chemotactic agent. Higher levels of IL8 were observed in patients with severe dengue fever but not in mild dengue fever[28]. Studies have also reported a correlation between elevated IL-8 levels and the severity of dengue[29, 30].

CCL5

Chemokine (C-C motif) ligand 5 also known as CCL5 is a chemotactic cytokine. It attracts monocytes, DCs, granulocytes, and leukocytes and can stimulate T cells during viral infection Upon activation, platelets also secrete CCL5 and fibrinopeptides which

helps in the replication and propagation of several viruses in the host[19]. A study has reported lower levels of CCL5 to be circulating in severe dengue patients than in other patients[31]. This can be a prognosis marker of severe dengue.

2.5 Cytokines in protection and in immunopathogenesis

Although the pathophysiology of severe Dengue is complicated and not well understood, but it is thought to involve an exaggerated immune response to the virus, leading to cytokine storms. Cytokines are essential for both immunopathogenesis and protection against Dengue virus (DENV) infection. From moderate Dengue fever (DF) to severe Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), the outcome of the infection depends on the ratio of pro-inflammatory to anti-inflammatory cytokines.

Uncontrolled cytokine production leads to vascular leakage, plasma leakage, and severe hemorrhagic symptoms. Excessive TNF-α, IL-6, IL-8, and IL-1β cytokines increase vascular permeability, contributing to DHF and DSS and also promote endothelial dysfunction and hemorrhagic manifestations (32). While anti-inflammatory cytokines IL-10 and TGF-β suppress protective immune responses, allowing viral persistence (33). In case of secondary DENV infection, non-neutralizing antibodies facilitate viral entry into Fc receptor-expressing cells, leading to enhanced viral replication. This triggers hyperactivation of immune cells and excessive cytokine release, contributing to severe immunopathology (34).

Cytokines act as a double-edged sword in Dengue infection. While they are essential for viral clearance, their dysregulated production can lead to severe immunopathogenesis. Understanding cytokine dynamics can help in developing targeted therapies to modulate immune responses and prevent severe complications in Dengue.

2.6 DENV Envelope and NS1

The majority of dengue-specific antibodies found in human sera bind to several DENV serotypes and are weakly neutralizing. Only a small portion of the total DENV-specific antibody response consists of antibodies that efficiently and precisely neutralise DENV. (22). Many studies have shown that most neutralizing antibodies recognize E protein of DENV. Domain I, Domain II, and Domain III are the three structural domains that make

up the glycoprotein E protein which are involved in host cell receptor recognition and attachment of virus to the host cell so, antibodies against E- protein has a great potential of early protection. The structure of domain III is an immunoglobulin-like module that is exposed and accessible on the virion surface (23). Studies using monoclonal antibodies (mAbs) has shown the presence of neutralizing epitopes in all three domains of envelope protein, however primary interaction of neutralizing Abs and surface exposed loop of D-III was seen. Identifying novel epitope on E-protein which will be conserved in all dengue serotypes would be a promising vaccine candidate.

DENV NS1 is a highly conserved 46kDa glycoprotein and contains 12 conserved cysteine (Cys) residues and two glycosylation sites (Asn-130 and Asn-207) (19). It is translated as monomer and glycosylated in the endoplasmic reticulum (ER). Inside the cell it exists in dimer form in the lumen of the ER where it takes part in viral replication and packaging. Infected cell secretes hexameric form of NS1 which can be found in the blood from the first day of symptoms. Secreted soluble NS1 is detected in patient serum during illness and it is used as indicator of DENV infection. The levels of NS1 does not always correlate with the onset of hemorrhagic fever, however high level of NS1 is often associated with increased severity of dengue infection. Secreted form exerts multiple function like protection of the virus from complement system, induction of pro-inflammatory cytokines and chemokines (20). Studies also have reported role of NS1 in the disruption of endothelial glycocalyx which increases permeability and results in vascular leakage and in severe case causes death. Antibodies against dengue structural proteins, such as Envelope and PrM/M, are only effective during the viremic stage. In contrast, antibodies against NS1 will demonstrate greater effectiveness as it can decrease viral replication by complement-dependent cytotoxicity (CDC) of infected cells and it is associated with clearance of viral NS1 thereby playing a protective role by preventing NS1-dependent enhancement of infection during the crucial phase. Since NS1 is not a structural protein, ADE will not also be induced by it (21).

With presence of all four DENV serotypes in the country, outbreaks have been reported in different regions of India in recent past with predominance of a serotype. In region where co-circulation of DENV serotypes and favourable climate of the vector species exits, point of care testing must be done for early detection of infection. A good point of

care test should also be able to differentiate between different serotypes. Insight study on T cell and B cell epitopes on DENV antigens could provide promising vaccine targets and detection assays that would be effective to all four DENV serotypes (24).

2.7 Disease management and the prospect of vaccine

Similar to the flu, dengue fever can affect both adults and small children and, in rare circumstances, result in death. Mild to severe dengue fever are among the clinical symptoms that the dengue virus can produce. Following a 4-6 day incubation period, patients with dengue fever may have symptoms such as headaches, vomiting, fever, stomach pain, and maculopapular rash. Documented indicators of severe dengue cases include severe bleeding, thrombocytopenia, vascular permeability, plasma leakage, and organ damage. Dengue fever does not yet have a documented cure or preventive measures, although infections can be managed by quickly diagnosing cases and starting rehydration therapy.

There are now two approved vaccines: Dengvaxia and QDENGA (Takeda). These are live attenuated tetravalent chimeric vaccines. QDENGA has an attenuated DENV-2 backbone with DENV1, 3, or 4 prM/E genes, whereas Dengvaxia has a 17D yellow fever vaccine virus backbone with prM/E genes of DENV1-4. Individuals between the ages of 9 and 45 who have had a prior DENV infection are advised to use Dengvaxia. Even though Dengvaxia worked well for seropositive individual, long-term safety data showed it increased the risk of more severe symptoms upon dengue infection in seronegative and in children below 9 years of age. There have been no significant side effects associated with the Qdenga vaccine, and it has been determined to be well-tolerated showing encouraging outcomes, as it is generally effective in preventing children and adolescents from developing symptoms of dengue. On the other hand, immunocompromised people and pregnant and lactating women should not use it [35-37].

Despite of these two approved vaccines, the efficacy is a major concern as efficacy of Dengvaxia for DENV 3 and 4 is reported to be 70-80%, and for DENV 1 and 2 40-50%, whereas efficacy for Qdenga seropositive 76.1%, seronegative 66.2% [38]. This is because serostatus of an individual greatly affects how well dengue vaccines work. Seropositives respond to the vaccine more strongly, whereas others who are seronegative might respond less well. In order for vaccines to be as effective as possible, they must

provide protection against all four of the dengue virus's distinct serotypes.

2.8 Reverse vaccinology approach in designing vaccine

Given the frequent pandemics and epidemics, there is a growing need to accelerate vaccine development. It is difficult to develop vaccines against some viruses because of a number of issues, most notably the incapacity to cultivate some viruses in cell cultures and the great variation in human MHC profiles. Reverse vaccinology (RV) has streamlined the vaccine development process by effectively overcoming these constraints and making it easier to identify epitopes from antigenic proteins throughout the whole proteome. Many benefits, like as broad proteome coverage, precise epitope identification, cross-protection capabilities, and MHC compatibility, are provided by the RV method in the development of accurate and potent vaccines against viral infections. However, RV-based vaccine candidates need to be experimentally validated to determine their protective efficacy for practical uses, regardless of how promising they seem [36].

Many researchers have used RV approach to design promising and effective peptide-based vaccines against dengue virus [40-42]. Antigenic, immunogenic, non-allergic, and conserved B and T-cell epitopes are usually identified using bioinformatics techniques. These potential epitopes are then joined together with linkers and adjuvants to construct the vaccine. This reverse vaccinology approach along with experimental validation is a promising technique for quick development of vaccines in the future.

2.9 References

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