Characterization of antibody and cellular response to Dengue virus infection to determine cytokine/chemokine markers of inflammation and serological diagnosis of Dengue virus infection

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CHAPTER VII: SUMMARY & CONCLUSION

7.1 Summary

Dengue also known as breakbone disease has become a major public health concern in most tropical and subtropical countries [1, 2]. It is transmitted through the bite of a DENV-infected mosquito. Mild symptoms of Dengue infection include fever, headache, body aches, and pain among others while severe forms of Dengue is associated with plasma leakage, thrombocytopenia, vascular permeability [3]. Due to its rise in incidence, it has become very important to control the disease before causing an outbreak.

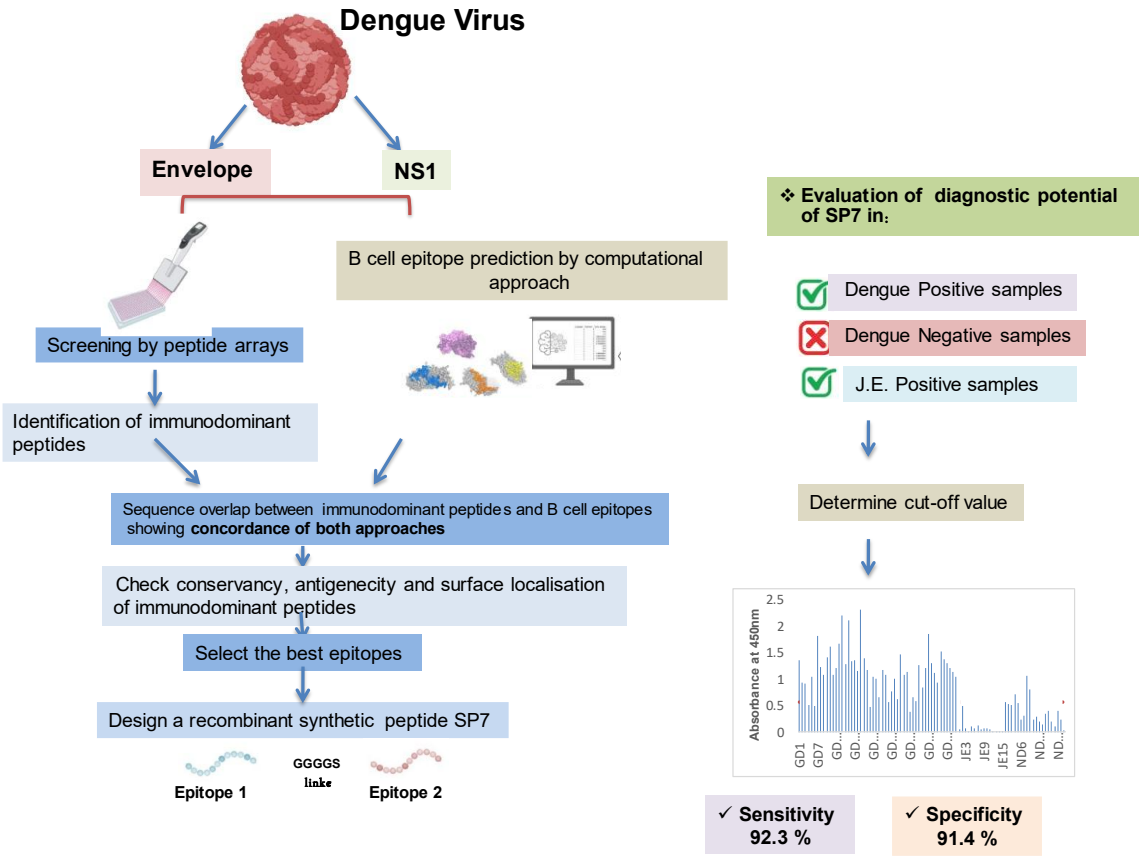

Figure 7.1 Schematic diagram of the selection of immunodominant epitopes of Dengue Virus Envelope and NS1 Proteins using both peptide array and computational approaches and evaluation of the diagnostic potential of the designed synthetic peptide SP7.

As there is no specific treatment for Dengue, proper management of the disease solely depends on early and accurate diagnosis. Therefore, it is very crucial to have a specific diagnostic test that can be employed for rapid diagnosis. Epitopes are now an evolving research area for use in diagnostics and therapeutics. We have focused our study on

identifying potential epitopes on the DENV envelope and NS1 proteins. Both peptide array and immunoinformatics approaches were used to identify epitopes of DENV proteins. (shown in Figure 7.1) Potential epitopes were screened based on seropositivity and best epitopes were selected to design a recombinant synthetic peptide. Evaluation of the synthetic peptide yielded more than 90% sensitivity and specificity which validates the diagnostic potential of the peptides.

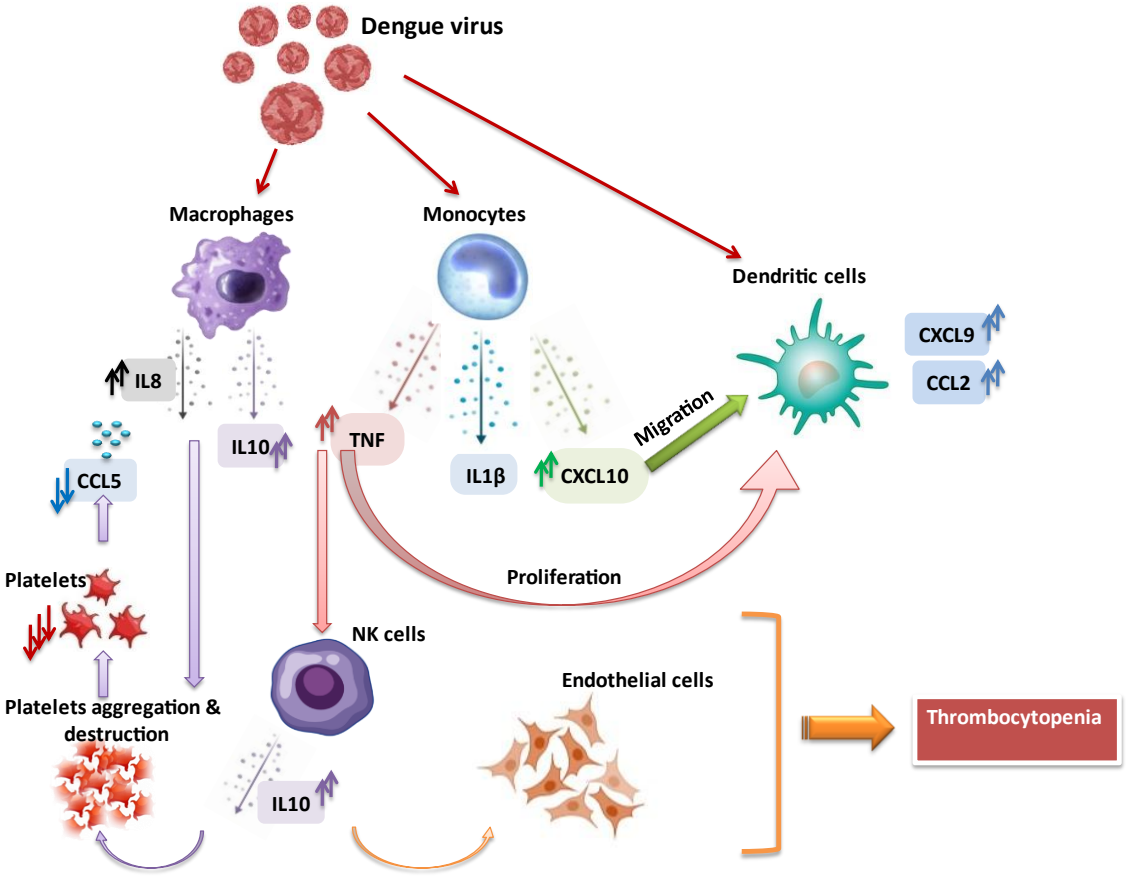

Figure 7.2: In Dengue disease, activated monocytes secrete TNF and IL-1β. The elevated levels of TNF promote the proliferation of dendritic cells. Additionally, the higher secretion of CXCL10 by monocytes aids in the migration of dendritic cells. This process also triggers the activation of NK cells and the release of IL-10, which targets endothelial cells and contributes to platelet aggregation, leading to thrombocytopenia in Dengue.

It is also very important to understand the dynamics of the pathogenesis of Dengue so that progression to severe Dengue can be controlled. Cytokine storm is pretty evident in Dengue infection. This phenomenon causes uncontrolled cell stimulation and altered

cytokine production causing vascular permeability and plasma leakage leading to dengue disease severity. Therefore, we investigated the serum cytokine and chemokine protein expression levels of Dengue-positive patients to explore insights into the pathogenesis of Dengue infection. Our findings reveal the activation of the monocyte-neutrophil axis in Dengue inflammation which is indicated by the increased levels of cytokines such as IL6, IL1β, and IL10 in Dengue patients in comparison to healthy individuals. Elevated levels of chemokines CXCL9, CCL2, CXCL10, and IL8 also promote the monocyte-neutrophil axis. However, in contrary to other chemokines we have found that expression of CCL5 was higher in healthy control than in Dengue-positive patients. Platelet cells being compromised in DENV infection [4] and a positive connection between platelet count and CCL5 levels observed in our study population suggests that low CCL5 expression might be a combined contribution from platelets and immune cells (Figure7.2).

7.2 Conclusion

Given the genetic makeup of individuals from the Northeastern part of India is distinct from Mainland India [5, 6, 7] , it was pertinent to study the severity of dengue in relation to key cytokines and chemokines in it. The significant role of the monocyte-neutrophil axis in mediating inflammation during Dengue virus infection is highlighted by our findings of higher levels of proinflammatory cytokines, along with increased levels of monocyte-neutrophil recruiting chemokines such as CXCL10, CCL2, and CXCL9. We also observed a predominance of CD14++ classical monocytes and intermediate CD16+ CD14+ monocytes in Dengue patients

Our results on the identification of potential peptides showed strong agreement between peptide array and bioinformatics approaches. This agreement confirms the diagnostic potential of these peptides. The diagnostic potential of in-house designed synthetic peptide when evaluated showed a sensitivity of 92.3% and 91.42% specificity. We have used a limited number of freshly collected samples for validation and it needs to be validated in a larger number of samples. This study supports that epitope-based antigens are promising candidates for the use in serodiagnosis of dengue.

The current work also demonstrated the rapidly developing reverse vaccine methodology that creates a multi-epitope based vaccine against the dengue virus, by using

immunoinformatics tools to examine T-cell and B-cell epitopes. This strategy has great promise for combating the increasing rate of infection and improving public health outcomes. Although the vaccine is found to be immunogenic, more research is needed to determine the precise level of immune protection against the virus. Although the results are still preliminary and require more investigation in vitro and in vivo, the selection criteria used for post-analysis and epitope filtering were very strict, which supports that the designed vaccine construct could be a strong candidate against Dengue virus. To ensure the safety and effectiveness of this vaccine candidate, however, further investigation and clinical studies are needed before it can be broadly implemented as a preventive strategy.

7.3 References

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