IMPACT OF DENGUE VIRUS ON INNATE AND ADAPTIVE IMMUNE SYSTEM
AMIT GUPTA, SUSHAMA R CHAPHALKAR
Department of Immunology, Vidya Pratishthan’s School of Biotechnology (VSBT) Vidyanagari, Baramati 413133, Pune, India

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Abstract: Dengue virus is a mosquito-transmitted single stranded RNA virus belonging to genus Flavivirus. Number of human cell types which is targeted by dengue viruses i.e. monocytes, macrophages and dendritic cells are the members of innate immunity which is capable of enhanced or rapid inflammatory responses. These innate immune cells are also major antigen presenting cells which is responsible for activating the adaptive immunity for long-term memory. In this paper, we discuss about the dengue negative and positive blood samples of human and studied its various immunological parameters i.e. CD3/CD4/CD8 population and peritoneal macrophages activation in mice. These studies directly or indirectly correlate with the innate as well as adaptive immune system.

Keywords: Dengue; Flavivirus; innate; adaptive
INTRODUCTION

The human body is the most complex type of immune system and it provides the primary defense against the dengue virus [1, 2]. If someone is infected with dengue infection or disease, our body immune system i.e. innate and adaptive work together to fight against the dengue virus or disease [3, 4]. Dengue disease is one of the most important insect-transmitted viral illnesses and is generally transmitted to human beings by the bites of Aedes aegypti or Aedes albopictus mosquitoes [5]. Every year, millions of people died due to dengue infection in all over the world. Currently, there is no vaccine available in India and abroad for the treatment of dengue disease. The treatment of this disease depends on severity which includes administration of intravenous fluids, blood transfusion etc. Generally, dengue disease in human observed in two phases i.e. dengue fever and dengue hemorrhagic fever. Till now, researchers identified four different serotypes in dengue disease i.e. DENV 1 to 4 [6, 7].

The occurrence of dengue disease has increased enormously due to the wide range of the virus and its mosquito vectors. One of the phase of dengue disease i.e. Dengue fever is due to 1 of 4 types, when a person recovers from the phase of dengue fever they develop a long-term immunity against that particular serotype, but not with other serotypes. The major symptoms which are appeared during dengue fever are pain (muscle, joint and abdominal), fever, erythema, low platelet count and viremia [8, 9]. If the same person is infected again with a different dengue virus serotype, they may develop the more severe form of the illness known as dengue haemorrhagic fever (DHF) and mostly affects on children [10, 11]. The major symptoms which are appeared during DHF infection are fluid build-up or produced in the lung as well as in chest cavities, liver failure, and brain damage. Although the two disease phases related to dengue virus can resolve in as little as two to three days, complete recovery can take weeks to months [12]. Now a day, DHF affects almost most of the Asian countries and has become a leading cause of death among children. Presently, there is no specific treatment or medication available for DHF. Normally, number of appropriate symptomatic treatment has been successful in reducing the mortality of DHF [11, 12]. In this review, it provides the information about the innate as well as adaptive immune system in dengue infection.

Correlation between innate and adaptive immune system with dengue disease

The innate immune represents the first line of defense towards infections but still under the control of adaptive immune system. One of the category of innate immune system i.e. macrophages (tissues) which are derived from monocytes (present in blood) and is generally grown in the bone marrow. Monocytes enter into the bloodstream, circulate all over the body and squeeze through the endothelium into tissues. Once in the tissues, they are called macrophages. Some monocytes differentiate into specialized cells such as dendritic cells,
Langerhans cells, Kupffer’s cells or microglia etc. Macrophages ingest the pathogens, digest them and present their antigens with major histocompatibility complex (MHC) class II molecules on their cell membranes to B lymphocytes and T cells to generate antigen-specific immune response. Macrophages again come into play as they opsonize the virus or the cells with antibodies attached to control and eliminate the virus [13, 14, 15]. One of the example i.e. dengue virus pathogenesis is very complex and multifactorial and macrophages which played an important role in disease both as primary targets of viral infection and as a source of immunomodulatory cytokines. The four serotypes of dengue virus (1-4) bind to a number of opsonic and non-opsonic receptors on cells of the mononuclear phagocyte lineage including DC-SIGN, glycosaminoglycans and when in complex with specific antibody, Fc and complement receptors. During dengue virus infection in mice the number of macrophages is reduced in the peritoneal cavity, bone marrow derived macrophages (using monocyte colony stimulating factor, m-CSF) and several functions are suppressed. These include reduction in the phagocytic activity of T dependent and independent antigen and reduced migration of peritoneal cavity of macrophages. Fc-receptor-mediated attachment and ingestion of opsonized sheep erythrocytes (EA) by the macrophages of spleen and peritoneal cavity are also adversely affected during DV infection in mice [14, 15].

Human negative and positive dengue blood samples were collected from Mangal Pathology Laboratory, Baramati, District Pune, Maharashtra, India at different time intervals for flow cytometric studies. Thirty dengue cases which are confirmed in Mangal Pathology laboratory, Baramati, Maharashtra, India on the basis of NS1 antigen to dengue virus; IgM and IgG antibody titre are not detected (Kit used J Mitra, Dengue NS1 antigen and IgM/IgG antibodies). One of the symptom is commonly observed in dengue disease i.e. thrombocytopenia is normally found in DHF. It was suggested that dengue virus showed enormous suppression in bone marrow ultimately leads to decline in platelet synthesis and resulted in thrombocytopenia. In dengue virus disease, serotype 2 can bind to human platelets in the presence of virus-specific antibodies, and an immune mediated clearance of platelets was proposed to involve in the pathogenesis of thrombocytopenia in DHF. It is already reported that IgM anti-platelet autoantibodies in dengue patients or in dengue virus infected mice. The titer of IgM anti-platelet antibodies is higher in DHF than in DF patients. The presence of these autoantibodies in dengue infected persons not only induces platelet lysis through complement activation but also inhibits ADP-induced platelet aggregation [16]. The antiplatelet antibody binding activity was observed only in dengue infected human patient samples and these activities are not observed in other virus-infected patient sera. In an attempt to inject (intraperitoneally, 10⁹ cells/ml) infected cells of dengue blood samples (lysed red blood corpuscles and wash two times with phosphate buffered saline) in mice, whole blood and peritoneal macrophages were processed for flow cytometric analysis at different time intervals. FACS and ELISA based assays were
utilized to evaluate and quantify the interactions and activation of these dengue samples. The *in vivo* experimental studies in mice were further verified with *in vitro* experiments using lymphocytes, monocytes and granulocytes isolated from healthy individuals. Furthermore, immune complex of negative as well as positive dengue virus samples of human binding to monocytes/macrophages and granulocytes may promote the secretion of inflammatory cytokines. In dengue virus, viral RNA binds to the pathogen-recognition receptors (MDA-5 and RIG-I) [17], which may further contribute to inflammation and cytokine production. A flow cytometric study of human whole blood positive and negative during dengue virus infection has shown early upregulation of granulocytes. This suggests that an increase in granulocytes may be occurring in patients at some stages of infection. Mice repopulated with human peripheral blood mononuclear cells are a powerful tool for the study of human dengue virus and immune function *in vivo* [18]. However, existing or well established humanized mouse models cannot support development of human innate immune cells, including myeloid (erythrocytes) cells and natural killer cells. Here we discuss about the Swiss mice, in which human peripheral blood mononuclear cells for innate as well as adaptive immune cell development are knocked into their respective mouse loci. This humanized mouse model based studies may be used to model the human immune system in scenarios of health and its pathological studies and should be further evaluation of therapeutic candidates in an *in vivo* setting relevant to human physiology [17, 18].

While the immune response to dengue viruses is classified or described in terms of CD4/CD8 population, CD3 surface marker and peritoneal macrophages activation in mice. The *in vivo* effect of normal as well as infected dengue blood samples was evaluated in Swiss mice. Mice were immunized intraperitoneally with normal as well as infected samples of dengue virus on day 0 and 7th day with human peripheral blood mononuclear cells (10⁹ cells) in a final volume of 0.2 ml. On day 4th and 10th day, collect the EDTA blood samples were used for the estimation of surface markers and peritoneal macrophages activation. For flow cytometric analysis, whole blood (100 µl, 10⁹ cells/ml) was taken from the retro-orbital plexus of mice in each tube. All these experiments will be done under the ethics rules and regulations. FITC labeled CD3, CD8 and PE labeled CD4+ monoclonal antibody were added directly to 100 µl of cells. Tubes were incubated in dark for 30 min at room temperature. Subsequently, 2 ml of 1× FACS lysis solution was added at room temperature with gentle mixing followed by incubation for 10 min. The samples were spunned (300 – 400 × g) and the supernatant was aspirated and sample was given three washings of PBS (pH 7.4). The resulting stained cell pellet was resuspended in 500 µl of PBS and was run on a flow cytometer. The forward and side scatter gating applied for data acquisition on 10,000 events in FACS Calibur and fraction of FSC and SSC cell populations representing different phenotypes analyzed using cell quest software [19, 20]. The activation of immunoregulatory T lymphocyte subsets (CD3 count) has been observed in dengue viral
infection, being more evident in mice than in human. There are, however, as yet no well-defined cell surface markers to determine the dengue viral infection will develop severe complications during the acute febrile stage of the disease. Number of studies was performed to compare the cellular immune status in mice in order to observe the value of these parameters in the immunization as well as challenging stage of the disease. The results showed that the first immunization with infected dengue virus samples showed slightly increase in CD3 count and there after second immunization with the same samples, there is rapid decline in CD3 count (Fig.1). In addition, cell mediated immunity which is mediated by T lymphocytes which played an important role to fight against intracellular or viral infections. Among the T lymphocytes, T helper cells induce B lymphocytes to secrete antibodies and cytotoxic T lymphocytes help phagocytes to destroy infection induced by pathogen and to kill intracellular pathogens or microbes. In this study, the infected samples of dengue in mice showed rapid increase in CD4 population at first immunization and then there is enormous increase in CD4/CD8 ratio as compared to control (Fig.2). Since mononuclear cells (especially monocytes) are over activated during acute dengue infection, it is expected or well established that increase in the activation of T cell markers such as soluble CD4, soluble CD8 and CD3 cell surface marker. These markers were much higher than DHF as compared to Dengue fever patients [7, 8, 9]. This clearly indicates the blood samples received from Mangal Pathology laboratory, Baramati, all are dengue fever samples of human whole blood.

In general, dengue-infected patients usually most of them are leukopenic for several days during the acute infection, characterized by a decrease in the absolute number of granulocytes and monocytes and these studies are confirmed through flow cytometric analysis as shown in Fig.3. The granulocyte or monocyte activation also contributes to the pathogenesis of DHF. Dengue virus stimulation induced monocyte activation in mice to increase the T cell surface marker i.e. CD3 (before challenging), CD4 and CD8 population (Fig.2). In case of dengue infected samples, there is reduction in CD3 T cell count after challenging injection (24 h) with respect to shape, size and granularity of the cell as compared to control which were immunized with negative dengue samples.

The macrophages from Swiss mice were collected from the peritoneal cavity and inoculated with infected dengue peripheral blood mononuclear cells of human on different time intervals. The macrophage cultures treated with the infected samples of dengue compared with untreated control cultures. The enhancing effect of the dengue peripheral blood mononuclear cells of human depended on the number of cells injected and duration of treatment. In flow cytometric based experiments, it was shown that the dengue peripheral blood mononuclear cells of human were found more frequently in treated cultures than in untreated control cultures. The data so far obtained suggest that the enhancing effect of peritoneal macrophages
activation was due to direct action of the dengue infected samples on macrophages. In short, peritoneal macrophages collected from Swiss mice and observed the cells through flow cytometer, it is confirmed that the infected dengue sample showed enhancement of peritoneal macrophages activation as compared to control (Fig.4).

Now a day, there is lot of progress in order to understand the mechanism and regulation of macrophage function, receptors and their signaling components for controlling the viral infection. With the advancement in the field of genomics, proteomics, immunology and availability of new technology it may be possible in near future to define the mechanism and regulation of macrophage activation and suppression.

CONCLUSION

Future studies should be taken into other infectious diseases including dengue as well. Inspite of the fact, we focused on adaptive humoral and cellular immunity in relation to medicinal plants to better address or understand the protective immunity.

Fig.1. Flow cytometric analysis of T cell surface marker i.e. CD3 in whole blood. Staining of cells with FITC labeled CD3 and then lysed with red cell lysis buffer and then wash the samples with phosphate buffered saline and then analyzed through flow cytometer.
Fig. 2. Effect of positive samples of dengue on CD4/CD8 ratio in EDTA mouse whole blood. EDTA blood was taken at different time intervals i.e. 4\textsuperscript{th} and 10\textsuperscript{th} day. Staining of cells with fluorescent dye with CD4 PE monoclonal antibody and CD8 FITC monoclonal antibodies and then analyzed the parameters through flow cytometer. Values are expressed as Mean ± S.E.

Fig. 3. Effect of positive samples of dengue on lymphocytes, monocytes and granulocytes on human whole blood. EDTA blood was taken and then lysed and washed the cells with phosphate buffered saline and then analyzed the parameters through flow cytometer.
Fig.4. Effect of positive samples of dengue on peritoneal macrophages. Macrophages were collected from the peritoneal cavity then analyzed the peritoneal cells through flow cytometer.

REFERENCES


