

Thesis Title           Evidence of Immune Cell Activation in Dengue  
                                  Hemorrhagic Fever/Dengue Shock Syndrome

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#### ABSTRACT

Peripheral blood mononuclear cell (PBMC) subpopulations, atypical lymphocytes and soluble factors in the serum were sequentially determined in 23 Thai children (3-12 years of age) with dengue hemorrhagic fever/dengue shock syndrome. There were 30 age-matched normal controls. The studies in patients were performed on febrile stage, day of shock or subsidence of fever (shock stage), the second and fourth day after shock or subsidence of fever (early convalescent stage) and once again at the recovery stage (approximately 14-21 days after shock or subsidence of fever).

The PBMC were stained by fluorescein isothiocyanate (FITC) or phycoerythrin (PE) labelled specific monoclonal antibodies to the surface antigens. Cells positive for specific surface markers were enumerated by a Fluorescence Activated Cell System (FACS) Analyzer. The result showed that the absolute total lymphocytes, CD3<sup>+</sup> lymphocytes, CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocyte subsets, B lymphocytes and NK cells were decreased at febrile and shock stage. Thereafter, all lymphocyte subpopulations were increased. The level of CD8<sup>+</sup> T lymphocytes and B lymphocytes rapidly increased above normal levels on the second day after shock or subsidence of fever, then gradually declined to the normal range. CD3<sup>+</sup> lymphocytes and CD4<sup>+</sup> T lymphocytes and NK cells increased gradually, CD3<sup>+</sup> lymphocytes and CD4<sup>+</sup> T lymphocytes reached their normal values on the second day after shock or subsidence of fever but NK cells still were in subnormal level through convalescent period.

Atypical lymphocytes were highest on the day of shock or subsidence of fever. The pattern of atypical lymphocyte response in peripheral blood of these patients was closely related with spontaneous incorporation of <sup>3</sup>H-thymidine by PBMC. This finding suggests that atypical lymphocytes were activated and actively synthesizing of DNA. Flow cytometric analysis suggested that atypical lymphocytes were of a heterogeneous nature. They may consist of all subpopulations of lymphocytes, including CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes, B Lymphocytes and NK cells.

Studies of soluble factors in serum showed that soluble interleukin-2 receptor (sIL-2R), soluble CD8 (sCD8) and gamma interferon (IFN- $\gamma$ ) were significantly increased during the course of illness. The sIL-2R levels peaked on shock stage and gradually declined thereafter—a pattern of response that resembled those of atypical lymphocytes and spontaneous proliferation of PBMC. The sCD8 levels peaked on the second day after shock or subsidence of fever. IFN- $\gamma$  levels were significantly increased at the febrile stage and rapidly declined on the day of shock or subsidence of fever.

The findings in this study provide evidence that all subpopulations of lymphocytes, including CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes, B lymphocytes and NK cells are activated during the acute phase of dengue infection. The findings also suggest that these cells may contribute to the pathogenesis of hemorrhagic phenomenon and shock which is the severe complication in DHF/DSS.