

Kinetics of CD4⁺ T-Lymphocyte in Dengue Virus Infection

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Abstract

Cellular immunity plays an important role in viral infection. The activation of effector T-lymphocytes and the release of cytokines define the course of dengue viral infection. CD4⁺ T-lymphocyte induces the immunopathology via various mechanisms; however, its kinetics in different onset of fever and disease severity are not fully understood. This study was conducted to observe the kinetics of CD4⁺ T-lymphocyte level among dengue infected patients and to obtain the absolute cell count and relative percentage of CD4⁺ T-lymphocyte. This was a descriptive study on thirty six patients who met the WHO-1997 criteria of dengue infection, hospitalized in Dr. Sardjito Hospital in the period of March to May 2009. The CD4⁺ T-lymphocytes were examined using the flow cytometer. The significance of median CD4⁺ T-lymphocytes among days were assessed using Kruskal-Wallis and Mann-Whitney SPSS 11 for Window. The absolute CD4⁺ T-lymphocyte was significantly different among days ($p < 0.05$) between DF and DHF patients, while the relative CD4⁺ percentage count was not different ($p > 0.05$). The absolute CD4⁺ T-lymphocytes count was low in the beginning of the disease course and increased from the 2nd day of fever to the normal level on the 7th day. In conclusion, DF and DHF do not differ in the absolute CD4⁺ T-lymphocytes count as well as in the relative CD4⁺ T-lymphocytes percentage. [MKB. 2014;46(4):221–24]

Key words: CD4⁺ T-lymphocyte, dengue fever, dengue hemorrhagic fever, dengue virus

Kinetik Limfosit T CD4⁺ pada infeksi Virus Dengue

Abstrak

Imunitas seluler berperan penting dalam infeksi virus. Aktivasi efektor limfosit T dan terlepasnya sitokin merupakan gambaran utama perjalanan klinis infeksi virus dengue. Sel limfosit T CD4⁺ menginduksi imunopatologi melalui berbagai mekanisme, namun bagaimana kinetiknya pada hari demam yang berbeda dan derajat berat penyakit masih belum sepenuhnya dipahami. Penelitian ini bertujuan untuk mengamati kinetika sel limfosit T CD4⁺ pada pasien infeksi dengue dan mendapatkan kadar absolut serta persentase relatif limfosit T CD4⁺. Penelitian deskriptif ini mengikutsertakan tiga puluh enam pasien yang memenuhi kriteria WHO 1997 untuk infeksi dengue, dirawat di RSUP Dr. Sardjito antara Maret sampai Mei 2009. Kadar limfosit T CD4⁺ diperiksa menggunakan *flow cytometer*. Perbedaan median limfosit T CD4⁺ pada berbagai hari demam dianalisis menggunakan Uji Kruskal-Wallis dan Mann-Whitney SPSS 11 untuk Windows. Kadar absolut limfosit T CD4⁺ berbeda secara bermakna antara hari demam ($p < 0,05$). Persentase relatif limfosit T CD4⁺ tidak berbeda bermakna antara demam dengue dan demam berdarah dengue ($p > 0,05$). Kadar absolut limfosit T CD4⁺ rendah pada awal perjalanan penyakit, meningkat pada hari ke-2 demam dan mencapai nilai normal pada hari ke-7 demam. Demam dengue dan demam berdarah dengue tidak menunjukkan kadar absolut maupun persentase relatif limfosit T CD4⁺ yang berbeda bermakna. [MKB. 2014;46(4):221–24]

Kata kunci: Demam berdarah dengue, demam dengue, limfosit T CD4⁺, virus dengue

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Introduction

Dengue infection is currently considered as one of the major global health problems. Around 50–100 millions of cases are estimated to occur annually in the tropical areas of the world. World Health Organization has categorized Indonesia, Thailand, Sri Lanka, and Timor-Leste in countries with the highest endemicity. Current estimation stated that at least 100 countries are endemic for dengue hemorrhagic fever (DHF) and about 40% of the world population (2.5 billion people) in tropic and sub-tropics region are now at risk for dengue infection.¹ In 2006, Indonesia contributed to 57% of dengue cases in Southeast Asia Region itself. Dengue morbidity increased during the period of 2003 to 2007. However, the number of reported cases has reached a plateau after 2007 up to 2009. Th case-fatality rate has decreased in the period of 2006 to 2008, but it has increased in 2009 to 2010.²

CD4⁺ T-lymphocyte cells may contribute to the host response to pathogens through different mechanisms including by releasing cytokines and mediating cytotoxicity. CD4⁺ T-lymphocyte can induce the production of chemokine which attracts CD8⁺ T cells to sites of infection. Studies on T cell immunopathology in animal models showed that CD4⁺ and CD8⁺ can cause damage when they are produced in high frequencies and high load of antigen.^{3,4} Frequencies of activated CD8⁺ T cells in the peripheral blood circulation that are relatively higher have been identified in patients with DHF, compared to those who suffer from Dengue fever (DF).⁵

Despite the known importance of CD4⁺T cells in the host response to pathogens, its kinetics in different clinical stages is still not well defined. Hence, we aimed to describe the kinetics of the CD4⁺ T-lymphocyte level among dengue infected patients.

Methods

Patients ≥14 years of age suspected for dengue infection based on WHO-1997 case definition, who were on the first three days of fever onset with positive NS1 antigen detection, and were hospitalized in Dr. Sardjito General Hospital, Yogyakarta between March and May 2009 were involved in this study. Informed consent was given by the patients or their guardians prior to blood specimen collection. Patients with co-infection other than dengue were excluded from this study. Blood samples were extracted from anterior cubital vein using sterile K3 EDTA vacutainer blood collection tubes and

were kept in room temperature (20–25 °C). The reagent used was Tritest CD3 FITC/CD4 PE/CD45 per CP and the results were analyzed in a FACS Calibur flow cytometer using the Multiset software. The CD4⁺ level from each patient was recorded from the second day to the seventh day of fever. Serological test was taken to confirm the diagnosis of dengue infection by using IgM and IgG. Demographic features of the patients were collected in this study based on gender, sex and diagnosis. Absolute CD4⁺ T-lymphocyte count and relative percentage of CD4⁺ T-lymphocyte were calculated and recorded for each patient. Descriptive analysis and Kruskal-Wallis test were done to obtain the kinetic of CD4⁺ T-cell from the second day of fever until the seventh day. The difference in amount of CD4⁺ T-lymphocyte between DF and DHF was calculated using Mann-Whitney test. Approval was taken from the Ethics Committee and Review Board of Universitas Gadjah Mada.

Results

As shown in Table 1, more male patients enrolled in the study. About half of the patients were ≤18 years of age. Out of thirty six, twenty subjects had been diagnosed with dengue fever (DF) and sixteen suffered from dengue hemorrhagic fever (DHF).

The pattern of CD4⁺ T-lymphocyte absolute count between DF and DHF based on day of fever is shown in Figure 1. There was a similar pattern of CD4⁺ T-lymphocyte absolute count between DF and DHF, in which both of them consistently increase from the beginning of the febrile phase

Table 1 Baseline Characteristics of the Patients

Characteristics	Frequency
Gender	
Male	23
Female	13
Age (years)	
14–18	18
19–29	16
≥30	2
Diagnosis	
DF	20
DHF	16

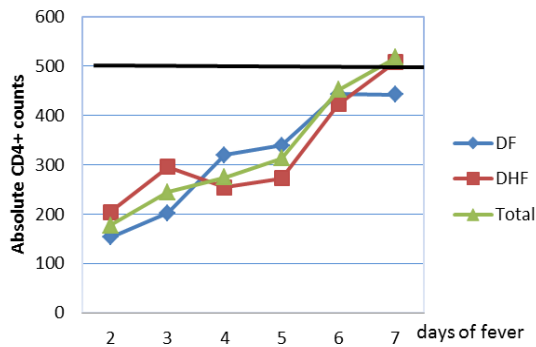


Figure 1 CD4⁺ T-lymphocyte Absolute Count for Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF)

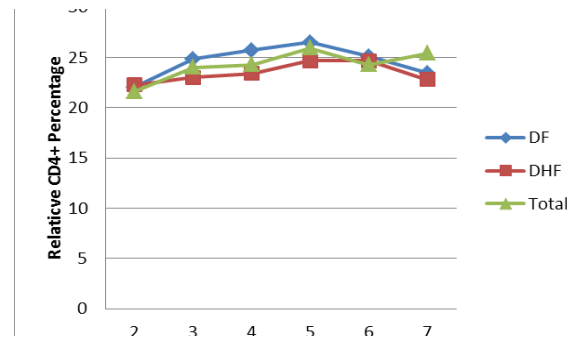


Figure 2 Relative CD4⁺ Percentage of Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF)

to the end of day 7. On the second day of fever, the average of total CD4⁺ T-lymphocyte absolute count was 177 cells/ μ L. It increased accordingly to 245 cells/ μ L on the third day, 313 cells/ μ L on the fifth day, and to 518 cells/ μ L on the seventh day.

In contrast, there was no significant difference in relative CD4⁺ percentage from day to day in both DF and DHF. The average relative CD4⁺ percentage was relatively constant from time to time and the mean ranged from 22% to 26%.

When a test was performed to analyze the median number of CD4⁺ T-lymphocytes, most of the results were not significantly different among DF and DHF patients, except for the relative percentage on day 7 ($p=0.022$).

Discussion

The CD4⁺ T-lymphocyte count has been known to be influenced by age, sex, race, time of specimen collection, drug administration, viral infection, and physical exercise. Compared to adults, the CD4⁺ T cell count in children is relatively higher. A study conducted among healthy Asian people reported a range of CD4⁺ cell counts starting from 401 to 1,450 cells/ μ L.⁶ Clearly, in the beginning of the disease course, the CD4⁺ absolute cell count was below the normal level. Our study found that absolute CD4⁺ T-cells was low in the beginning but gradually increased from day 2 of fever to its normal level at day 7. The absolute CD4⁺ T-lymphocyte count differed significantly among days of fever ($p<0.05$). Unlike our study, Azeredo et al.⁷ reported that the mean percentage of CD4⁺ T-cells was reduced during acute phase of dengue infection and its absolute cell counts decreased when compared to controls. This did not return to normal level until the convalescence

period. Meanwhile, a study that was conducted by Mabalirajan et al.⁸ showed that around the time of fever defervescence, the CD4⁺: CD8⁺ ratio was significantly lower in patients with dengue infection than in healthy people. Another study also reported that after the CD4⁺ T-lymphocyte decreased in the early course of disease, it would gradually increase and returned to its normal absolute level on the second day after the time of fever defervescence or after shock.⁹ Another suggestion have been raised recently that the CD4⁺ T-lymphocytes increase gradually until the 14th day post-infection, deducted by the incubation period, that it would be apparent on day 7 after the clinical manifestation.¹⁰

The mean relative CD4⁺ percentage in this study was ranging from 22% to 25% and was statistically insignificant ($p=0.573$). The percentage of CD4⁺ T cells, unlike the absolute CD4⁺ T cells count, is relatively stable in terms of the time of the day, reagents and biological factors influencing the absolute CD4⁺ T cells count.⁶ Several other studies have found that those patients with relatively high absolute CD4⁺ T lymphocytes count but low CD4⁺ T lymphocyte percentage have faster disease progression when they are compared to persons with low absolute CD4⁺ T lymphocytes count but high percentage of CD4⁺ T lymphocytes.^{11,12} The results showed that absolute CD4⁺ T-lymphocytes counts have no significant difference in term of the severity of the cases. Several studies have pointed out Flavivirus-cross-reactive CD4⁺ T-cell activation in heterologous infection and have suggested its contribution to the severity of disease and to a comparable difference in absolute CD4⁺ count among mild and severe dengue infection cases.¹³ This may probably be due to the lower threshold of memory T-lymphocytes when compared to the naive cells or the low affinity towards the second serotype which leads to delay in viral clearance

and immunopathology.¹⁴ The increasing amount of CD4⁺ T-lymphocyte may be correlated with the response of the secondary infection when the cells were exposed to the heterologous antigens, which then produced significantly higher amount of cytokines. A study by Yauch et al.³ in an animal model found that although CD4⁺ T-lymphocyte does not have any contribution in controlling DENV2 primary infection, by using epitope identification, there was a significant role of CD4⁺ T-lymphocyte in the secondary homologous and heterologous infections.

In conclusion, absolute CD4⁺ T-lymphocyte count is low in the beginning of the disease course and increases from day 2 of fever to the normal level on day 7 of fever. The relative CD4⁺ T-lymphocyte percentage shows no significant differences in increment. No significant difference in the absolute CD4⁺ T-lymphocyte count and relative CD4⁺ T-lymphocyte percentage is found between dengue fever and dengue hemorrhagic fever.

Further studies involving longer follow up period and other lymphocytes are required to have a more integrated perspective on the kinetics of CD4⁺ T-lymphocytes in dengue infection.

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