The HLA-DR Expression on Monocytes in Acute Dengue Infection

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ABSTRACT

Introduction: Dengue is the most common disease of mosquito-borne infection. According to the World Health Organization[7], it is estimated that 50 million cases of dengue infection have been reported annually. Macrophages and monocyte are thought to play an important role in dengue infection both as primary targets of viral infection and as a source of immunomodulatory cytokines. When monocytes are infected by dengue viruses, it processed the virus by lyses it and expressed the antigen on its surface membrane bind together with HLA class II molecules. HLA-DR-expressing monocytes in acute dengue patients especially the intensity is still poorly understood. Measurement of at difference days of infection will give an evidence about the role of monocytes in dengue pathogenesis

Objectives: To observe the kinetics on percentage of HLA-DR-expressing monocytes and the median of HLA-DR expression intensity in acute dengue infection.

Methods: This research was an observational type study conducted by cross sectional method towards all the dengue patients in Dr. Sardjito's General Hospital. Blood samples were drawn from 32 acute dengue infected patients from Day 1 to Day 6th. The HLA-DR expression was measured flow cytometrically using FACS Calibur.

Results: The data showed that the mean difference in acute dengue infection from Day 1 to Day 6 is not significant with the p-value larger than 0.05, ($p>0.05$).

Conclusion: The peak level of activated HLA-DR monocyte was at day 2 and then decreasing until day 6. There were no significant changes in percentage of HLA-DR-expressing monocytes from the day 1 to the day 6 and the median intensity of HLA-DR expression in acute dengue infection.

Keywords HLA-DR-expressing monocytes, Acute Dengue Infection

INTISARI

Pendahuluan: Infeksi Dengue adalah penyakit tersering dikalangan penyakit yang mengunakan nyamuk sebagai vektor. Diperkirakan 50 juta kasus infeksi dengue terjadi setiap tahun. Makrofag dan monosit memainkan peran yang penting dalam infeksi dengue sebagai target primer bagi infeksi dan juga sebagai sumber sitokin immunomodulator. Apabila monosit terinfeksi oleh virus dengue, monosit akan memproses virus tersebut dan mengekspresi antigen pada permukaan sel bersama molekul HLA kelas II. Pengukuran aktivasi monosit pada infeksi dengue akut akan memberikan informasi mengenai peranan monosit dalam patogenesis infeksi dengue.

Tujuan: Tujuan penelitian ini adalah untuk mengobservasi kinetic dari persentasi monosit yang teraktivasi

melalui ekspresi HLA-DR pada demam denggi akut hari pertama hingga hari keenam.
Metode: Penelitian ini dilakukan dalam metode penelitian observational secara cross-sectional.
Hasil: Data menunjukkan bahawa perbezaan purata pada hari-hari demam denggi adalah tidak signifikan dengan nilai p diatas batas 0.05, (p>0.05).
Simpulan: Secara keseluruhannya, antara hari pertama hingga hari keenam, secara statistiknya menunjukkan bahawa tiada perbedaan yang signifikan pada persentasi monosit yang teraktivasi.

Kata kunci: Ekspresi HLA-DR pada monosit, Demam Dengue Akut

INTRODUCTION

Dengue is the most common disease of mosquito-borne infection. According to the World Health Organization[1], it is estimated that 50 million cases of dengue infection have been reported annually. The prevalence of dengue has grown dramatically in recent decades. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia and the Western Pacific.

Dengue fever is an acute infectious disease caused by four serotypes of dengue virus (DV). It is characterized by biphasic fever, myalgia, headache, and pain in various parts of the body, rash, lymphadenopathy, and leucopenia[2]. Dengue fever is self-limited, but, it will progress to DHF or Dengue Shock Syndrome (DSS) in certain conditions, which is heterotypic infection of DV. Dengue hemorrhagic fever is a severe febrile illness characterized by abnormalities of homeostasis and increased vascular permeability, and severe progression may result in DSS. Dengue shock syndrome is a form of hypovolemic shock that is associated clinically with hemoconcentration and which might lead to death if appropriate care is not given. The other characteristic features of DHF include that the increase in vascular permeability is without morphological damage to the capillary endothelium, altered number and functions of leucocytes and increase hematocrit[4].

After DV infection, there is a continuum from mild DF to severe DHF or DSS. It has been estimated that only 4-6% of individuals with secondary infection develop severe DHF disease[4].

Monocytes appear to be important in the pathogenesis of dengue infection. They are thought to be the most active sites of virus replication during dengue infection. Monocytes 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. Monocytes again come into play as they opsonize the virus or the cell with antibodies attached to control and eliminates the virus.

A number of pathogens, including DV, subvert monocytes and use them for their replication increasing severity of the damage to body[3]. The extent of DV replication during the early periods of infection may determine clinical outcomes, which may be from asymptomatic infection to febrile illness, DF, to life-threatening disease, DHF/DSS. The impact of DV infection on innate immunity may be the determining factor.

The cells of macrophage-lineage, interstitial dendritic cell (DC) and Langhans cell (LC) constitute the first line of the innate host defense against invading DV in skin where it replicates after the initial bite by infected mosquito. When monocytes phagocyte a dengue virus, they process the virus by lyses it and then transported the lyses peptide to the surface of cell membranes and expressed it at the MHC class two molecules.

The complex of MHC class two molecules and dengue viral peptides will then be present to T and B cells so then an adaptive immune system will be initiated. The expression of HLA-DR in monocytes therefore is important as a marker for the HLA-DR-

expressing monocytes. Measurement of HLA-DR-expressing monocytes at difference days of infection may help in prognosis of dengue disease and may be correlated with severity of the disease.

RESEARCH METHOD

This research was designed as observational cross sectional. 32 patients who are under treatment at the internal medicine's department in Dr. Sardjito Hospital, Yogyakarta, were recruited as the subjects of the study. The inclusion criteria of the research subjects were patients who were suspected of having dengue fever with NS-1 positive test and the age more than 14 years.

The exclusion criteria of the research subjects were those who have chronic disease other than dengue infection. Patient's who fulfilled the inclusion criteria and did not have any of the exclusion criteria, were given informed consent and the procedure in this study was explained to them. After they agreed and signed the informed consent form, 3 ml of intravenous blood sample was taken aseptically from Day 1 to Day 6. The blood sample was kept in a room temperature between 20° C – 25° C. The sample was sent to the Pathology Clinic Laboratory for complete blood test using the hematology analyzer and analysis of HLA-DR activation marker was done by flow cytometry test.

For flow-cytometry examination, for every specimen, 50 uL of whole blood was inserted into a tube (12 x 75mm), EDTA anticoagulant with the correct amount was added. Ten uL CD HLADR PE / CD45 per CP antibody which is fluorescently labeled was added. Mixture was vortex slowly and incubated for 15 minutes in a dark room at the temperature of 20° – 25° C. Fifty uL 10x FACS Lysing Solution was diluted with 450 uL aquadest. 450 uL 1x FACS Lysing Solution was added to lyses the erythrocyte. Mixture was vortex slowly and incubated for 15 minutes in a dark room at the temperature of 20° – 25° C. The sample was analyzed using the FACS Calibur Flow cytometer. Population of monocytes was determined by the cell granulation and expression of CD 45.

Distribution of data was determined by using Probability-probability test. Descriptive statistical analysis was done for each day of acute dengue infection which include mean, standard deviation and minimum and maximum percentage of HLA-DR-expressing monocytes. Means difference in percentage of HLA-DR-expressing monocytes was calculated by using ANOVA. If the p-value is below 0.05, $p<0.05$, the mean differences is significant.

RESULTS AND DISCUSSIONS

From this study, out of 32 subjects, 17 subjects were classified as having dengue fever (DF) and the rest, 15 subjects classified having dengue hemorrhagic fever (DHF). This classification is done by using WHO guidelines of dengue infection. Table 1 shows the classification of dengue patient in this study.

Table 1. Classification of Dengue Infection in subject

Type of Dengue	Frequency (n)	Percentage (%)
DF	17	53.1
DHF	15	46.9
Total	32	100

The distribution of data in this study is normal from day 1 to day 6. Figure 1 is the probability-probability plot for day 1 that represents the normal distribution of data for day 1.

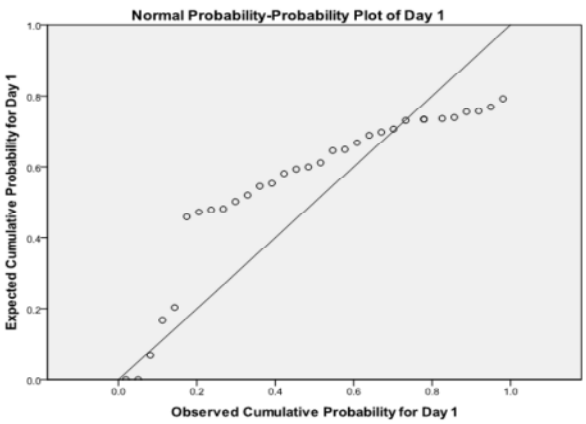

Figure 1. The Probability-Probability Plot of Day 1 on Percentage of HLA-DR-expressing monocytes

Table 2. Descriptive Analysis of HLA-DR-expressing monocytes

Day	Minimum (%)	Maximum (%)	Mean (%)	Std. Dev. (%)
1	68.29	99.43	93.27	7.55
2	66.67	99.52	92.69	7.71
3	52.38	99.38	90.15	9.46
4	76.27	99.12	93.17	5.27
5	60.00	98.32	92.43	7.34
6	50.00	98.11	90.06	8.68

Table 2 shows the minimum, maximum, mean and standard deviation of HLA-DR-expressing monocytes from Day 1 to Day 6 of acute dengue infection. The maximum percentage of HLA-DR-expressing monocytes was in Day 2, which is 99.52%. However the difference is not significant compare to other days, for example at Day 1, the maximum percentage of HLA-DR-expressing monocytes was 99.43% and it differs only 0.09%. While for the minimum percentage of HLA-DR-expressing monocytes was in Day 6 which is 50.0%, and the difference is big compare to other days thus, produce the highest standard deviation for Day 3 compare to other days of infection. For the mean, percentage of HLA-DR-expressing monocytes was lowest at Day 3 and highest at Day 1. From table 2 also, there is a trend that shows a decreasing percentage of HLA-DR-expressing monocytes from Day 1 to Day 3, which is from 93.27% to 90.15%. The percentages of HLA-DR-expressing monocytes increased again at Day 4 which is 93.17%. For the rest of Day 5 and Day 6, the percentage of HLA-DR-expressing monocytes slightly decreased.

For means comparison, the mean difference between days of infection is not significant with p value more than 0.05, (p>0.05).

Overall percentage of HLA-DR-expressing monocytes in acute dengue infection from our study is around 90% to 93%. This percentage is very high compare to our control negative subject which is only 22%. It means that there is a very rapid activation of monocytes during acute

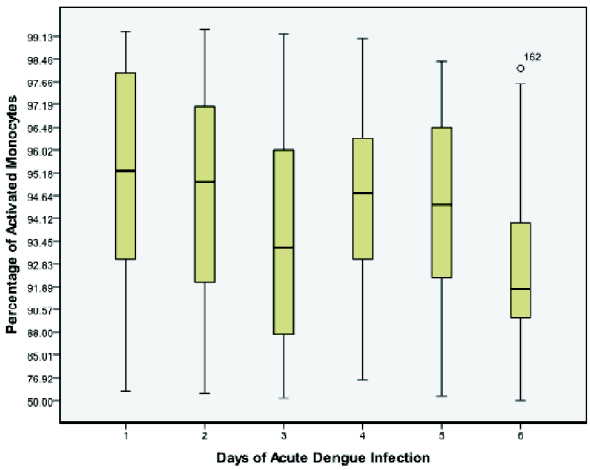

Figure 2. Percentage of HLA-DR-expressing monocytes Activated during Acute Dengue Infection

dengue infection. We suggest that this condition happen due to the immunopathogenesis of this disease itself. Many studies have demonstrated that monocytes are the target cells for dengue viral replication. Chaturvedi[4] (2006) has shown that the dengue virus replication rate is higher in monocytes compare to peripheral B cells. When monocytes were infected by dengue virus, they become activated. So, the activation of monocytes is rapid because the dengue virus attacks the cells itself. Means the activation of monocytes are directly related to dengue virus infection or dengue virus severity.

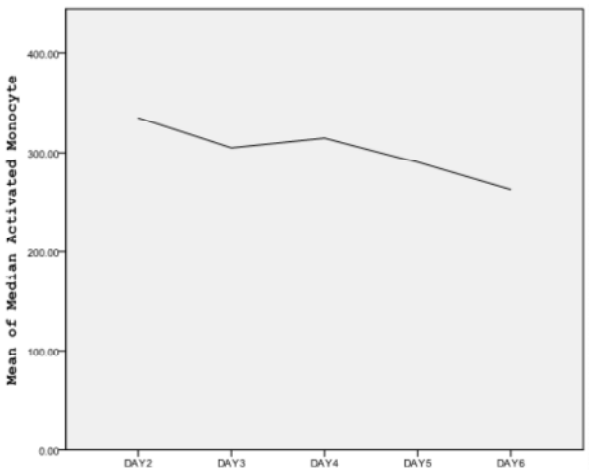

Figure 3. The Mean of Median Activated Monocyte

From figure 3 there was a decrease of data(mean) from day 2-3. An increase from day 3-4 and decrease after that from day 4-6. The highest amount of HLA-DR activated monocyte is at day 2. The analysis of variance (ANOVA) is done since we want to see the difference of HLA-DR activated monocyte for multiple days from day 2-6. From the median descriptive statistic we can see at day 2 there is a peak amount of activated monocyte. This is in line with a research which said that maximal infection rate was measured for wild type Asian genome (D2/IC-30P-A) at 48 h post infection[7]. They also uses flowcytometry HLA-DR to detect the manocyte derived macrophage[7].

Activation of monocytes by expressing HLA-DR molecules on its surface membrane and by releasing several cytokines is needed as part as our immune response to infection. One of the functions of monocytes is they serve as antigen presenting cell (APC)[8]. They present exogenous peptides processed as HLA-DR and bind it to the T cell receptors (TCR) of T cells. In a normal immune response, when there is a systemic infection, a large numbers of monocytes need to be activated. If there is low percentage of HLA-DR-expressing monocytes, or the expression of HLA-DR by monocytes is less in a systemic infection or systemic inflammation process, it will lead to poor outcomes of the patient. It has been proven by Haveman[6] (2006) that HLA-DR expressed by monocytes has been decreased in non-survivors patient with systemic inflammation process. In patients who have multiple organ failure and having severe inflammatory process reaction, the level of HLA-DR express by monocytes is decreasing[6]. It is then suggesting that in normal physiological condition where our immune response is capable to act against infection and inflammatory response, it is therefore a normal to have a high level of HLA-DR-expressing monocytes or increasing level of HLA-DR-expressing monocytes. This condition has also been supported from a research by Haveman[6] and colleague that shows the patient who increase expression of HLA-DR in systemic inflammation survive the condition.

The expression of HLA-DR by monocytes is regulated by certain mediators such as interferon-ã (IFN-ã) and tumor necrosis factor-á (TNF-á). There could be a more details explanations in molecular levels on how immunopathogenesis of dengue can influence the expression of HLA-DR in monocytes. Birle[3] (2003) have stated in his journal that TNF-á can down regulate the expression of HLA-DR in monocytes. Green[5] (1999) demonstrated that the level of TNF-á was different in DF and DHF. The TNF-á level in DHF was higher compare to DF. In DHF, the level of TNF-á was high at beginning which is at day 1 and decrease steadily from day 2 to day 6 compare to DF, the level of TNF-á is low at day 1 and highest at day 3 and decrease after day 3.

For the mean comparison, our study shows that there is no significant in mean difference between days of acute dengue infection. If we see from the data, there are no big changes occur in the percentage of HLA-DR-expressing monocytes from Day 1 to the Day 6 of acute dengue infection. The mean on percentage of HLA-DR-expressing monocytes throughout the six day of infection are around 90% to 93%. We suggest that the percentage of HLA-DR-expressing monocytes will remain high during acute period. Our study has limitation because we did not observe the factors or mediators that may affect the expression of HLA-DR in monocytes. This condition also related with a study from Green[5] (1999) that shows IFN-ã increase rapidly during early period of dengue infection and remain high for several days before it decrease suddenly.

Birle[3] (2003) in his article stated that IFN-ã increase expression of HLA-DR in monocytes. The level of IFN-ã in dengue infection was similar with the levels of TNF-á. It increase rapidly at day 1 and reach maximum level at day 3 and decrease after day 3. It may suggest that maybe the high concentration of IFN-ã at day 3 will cause the expression of HLA-DR increase at day 4, but it is not significant. However, this opinion is contradicted to previous explanation because the levels of TNF and IFN reach highest point at same day which was at day 3. Therefore,

we suggest that IFN and TNF alone cannot be a standard to monitoring the levels of HLA-DR expression in monocytes. There could be other explanations that can show on how HLA-DR is regulated.

CONCLUSION

Overall, it is statistically proven that there are no significant changes in percentage of HLA-DR-expressing monocytes from the day 1 to the day 6 in dengue infection. Percentage of HLA-DR-expressing monocytes in dengue infection remains high during acute dengue infection.

SUGGESTION

There could be more explanation on kinetics of HLA-DR-expressing monocytes if the researcher also observed factors that can affect the expression of HLA-DR molecules.

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