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Original Research Article

BALB/c Mice as Animal Model in Dengue Infection Research: Role of Endothelial Activation

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Abstract

Introduction. There were various challenges in using experimental animals model for dengue infection studies aside from the fact that dengue infection only naturally affects humans and does not manifest clinical signs as in humans. Various experimental animals have been used in dengue research, but the mouse model is more widely used since it is easier to obtain although sometimes they do not show clinical symptoms but may still measure the immune response. BALB/c mice are immunocompetent mice that have the potential to be used in dengue infection research. Endothelial cell activation plays a role in the pathogenesis of dengue virus infection which contributes to plasma leakage. One of the biomarkers of endothelial cell activation is soluble intercellular adhesion molecule 1 (sICAM-1).

Method. An analytic observational study was conducted using BALB/c mice aged 8 weeks and weighed 40 grams. Selected BALB/c mice were randomly assigned to serotype 2 dengue virus containing 2.1 x 10^6 pfu/ml intraperitoneally, given only once. A total of 11 mice were injected with dengue virus serotype 2 and 11 mice were not injected with dengue virus. On the second day of virus injection, non structural (NS) 1 antigen dengue examination was carried out to prove that the BALB/c mice were indeed infected with dengue virus. In BALB/c mice that were proven to be infected with dengue virus, sICAM-1 levels were examined in serum after 7 days of infection. Mice that were not injected with dengue virus were also examined for sICAM-1.

Results. All of BALB/c mice injected with dengue virus were proven to be infected, as indicated by the detection of NS1 antigen in their serum. The mean serum level of NS1 antigen was 88.35 ng/ml (mean 95.34 ng/ml and standard deviation 21.94). The level of sICAM-1 in BALB/c mice infected with dengue virus (mean = 1.34) was significantly higher than mice that were not infected (mean = 0.79), with a p-value 0.045

Conclusions. BALB/c mice were proven to be infected with dengue virus by detecting NS1 dengue virus antigen in the serum. The sICAM-1 levels in the group of BALB/c mice infected with dengue serotype 2 were significantly higher than the BALB/c mice that were not infected with dengue virus.

Keywords: *BALB/c mice; NS1 antigen dengue; sICAM-1*
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INTRODUCTION

Dengue virus infection is still a major public health concern in many countries, particularly in tropical areas like Indonesia.

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Dengue infection is endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia, and the West Pacific. In the last few decades, the incidence of dengue virus infection has dramatically increased, leading to significant morbidity and placing a socioeconomic burden on society. The clinical spectrum of dengue infection varies significantly from asymptomatic, undifferentiated fever, dengue fever, dengue hemorrhagic fever, dengue shock syndrome (DSS), and atypical manifestation (unusual manifestation/ expanded dengue syndrome). The pathological process that occurs is plasma leakage from the intravascular and the presence of hemorrhage.[1,2]

Manifestations of intravascular plasma leakage are strongly associated with changes in vascular function including the role of endothelium and other supports such as perivascular muscle cells, extracellular matrix, basement membrane, and glycocalyx. Dengue infection activates endothelial cells although the morphological changes are relatively minimal. Several biomarkers indicating endothelial cell activation were found to increase during dengue infection such as soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule (sVCAM-1).[3] Endothelial cells infected with the dengue virus were found in autopsy samples of Dengue Shock Syndrome (DSS) patients as well as in animal study models. it is suspected that these endothelial cells directly contribute to pathogenesis through increased viremia, cytokine secretion, complement modulation, and serving as targets of humoral and cellular immune responses. Dengue virus and TNFα together stimulate endothelial cell apoptosis through the production of reactive oxygen species (ROS). Through the role of vascular endothelial cells in the pathogenesis of dengue infection, a therapeutic approach by improving the vascular barrier through endothelial cells that can reduce plasma permeation is an option.[4]

The use of experimental animals in dengue infection research has faced many challenges, due to dengue infection does not naturally infect non-human species. Various animals have been tested in dengue research in terms of pathogenesis research, anti-viral, and development of dengue vaccine. Mice are the animal species most frequently used in dengue trials. Immunocompetent mice are naturally resistant to dengue virus but infection can occur with high levels of virus inoculation (10^6-10^8 pfu/ml). Previous study showed after administration of dengue virus serotype 2 as much as 10^8 pfu/ml intravenously to C57BL/6 mice, virus content in serum, spleen, liver, and brain can be detected which is marked by the spread of the virus and viral replication in various tissues.[5,6] Immunocompetent mice were also infected with dengue virus serotype 2 with a titer of 10^8 pfu/ml intravenously. Viremia was detected by RT-PCR on the second day after the virus injection.[7] Experimental studies using BALB/c mice that were injected intraperitoneally with dengue virus serotype 2 resulted in liver damage characterized by a progressive increase in SGOT and SGPT that peaked on the seventh day. Furthermore, dengue virus antigen was detected in hepatocyte cells by RT-PCR. BALB/c mice are susceptible to serotype 2 dengue virus infection, there are also structural and histological changes in the lungs of infected mice.[8,9] On the second to the eleventh day since infection with dengue virus serotype 2, BALB/c mice developed viremia. Dengue virus antigen was detected in the hepatocyte and the capillary endothelium of the central lobular vein area.[10]

OBJECTIVE:

This study aimed to support that BALB/c mice can be used as animal models in dengue virus infection research and to prove the existence of endothelial cell dysfunction in BALB/c mice infected with dengue virus serotype 2

MATERIALS AND METHODS

The research design was analytic experimental using BALB/c mice. Male BALB/c mice were reared until they were 8 weeks old and weighed 40 grams Mice were kept in a comfortable environment with 12 hours cycles of light and darkness and a room temperature of 25°–30°C. The cage was 30 x 40 cm in size with a 20 cm height, and there were 4 to 6 mice in each cage. Mice were fed daily with poultry feed with an amount of approximately 10-15 grams, or as much as the mice could consume. Then the randomization of the BALB/c mice was carried out to be used as a sample. At the end of the study, mice were euthanized as accepted in animal ethics. The mice were anesthetized with combination of ketamin, zylasine, and acetopromasin (mouse cocktail). Mouse cocktail were injected intramuscularly into the thigh muscle as much as 0.02 cc (200 microliters). Mice were euthanized by dislocating their neck bones then freezing them before burning them in an incinerator. Dengue virus serotype 2 isolates were obtained from the serum of dengue patients from the Denpasar site, AFIRE study by Ina Respond (Indonesia Research Partnership on Infectious Diseases). Isolate production was carried out at the Ministry of Health's Research and Development Laboratory, Jakarta. BALB/c mice were randomized, 11 mice were injected with dengue virus serotype 2 and 11 mice were not injected with dengue virus. In the selected BALB/c mice, dengue virus serotype 2 with a viral titer of 2.1 x 10^6 pfu/ml was administered intraperitoneally, given only once. On the second day of virus injection, NS1 dengue examination was carried out to prove that the BALB/c mice were indeed infected with dengue virus. Serum sICAM-1 levels by ELISA method were evaluated on the seventh day after infection using ELISA method in both groups. ELISA method product by BIOENZY catalogue number BZ-08188140-EB. Standard curve range 0.05ng/ml – 20ng/ml with sensitivity 0.026ng/ml.

RESULTS

All mice injected with dengue virus serotype 2 proved to be infected as indicated by the detection of NS1 dengue in the mice serum with varying levels as shown in the table 1:

Figure 1. BALB/c mice were injected with dengue virus serotype 2

Figure 2. Serum specimens were collected from BALB/c mice

Table 1. NS1 dengue level in the mice serum

Subject	NS1 **dengue level (ng/ml)**	Subject	NS1 **dengue level (ng/ml)**
1	96,932	**7**	98,864
2	77,121	**8**	101,780
3	97,538	**9**	58,438
4	95,985	**10**	99,735
5	102,159	**11**	96,439
6	94,621		

The mean serum NS1 dengue level was 88.35 ng/ml (median 95.34 ng/ml and standard deviation 21.94). There were no clinical symptoms in all mice that infected with dengue virus serotype 2.

The sICAM-1 levels in mice dengue infected was higher than the sICAM-1 levels in non-infected mice. The serum sICAM-1 levels in the two groups (the group infected with dengue virus and those not infected) appeared different as shown in the following graph:

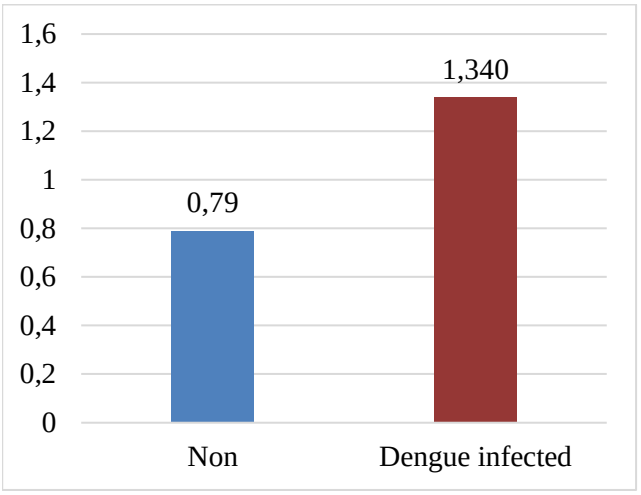

Figure 3. Graph of the serum sICAM-1 levels in mice non-dengue infected and mice dengue infected

DISCUSSION

Various animal models have been tested in research on dengue virus infection both in terms of pathogenesis, host immune response, development of dengue vaccines and anti-virus, but none of animal models have demonstrated clinical dengue symptoms as in humans.[6] Animal models that have been used in research on dengue infection include mouse models, human primate models and shrew models. Each animal model has its own advantages and limitations, such as level of viremia or viral replication, clinical manifestations and immune response, so the selection of an animal model depends on the purpose of the dengue infection research.[5,11] In recent years, studies on dengue infection have increasingly used mouse models because they are easier to obtain and can measure the immune response, however not all dengue-infected mice show clinical manifestations. Mice were infected with dengue by intraperitoneal, subcutaneous, intradermal or intravenous administration of dengue virus with an infective dose of 10^6 to 10^8.[5,6] The mouse model used could be immunocompetent mice or immunodeficient mice.[12,13]

BALB/c mice are immunocompetent mice which are also used in dengue virus research because they are proven to stimulate an immune response. [12,13] Various studies have shown that BALB/c mice are the most susceptible to dengue virus serotype 2 infection so that they can be used as a model in dengue research, especially in pathogenesis. In the study of dengue virus infection in BALB/c mice, viremia was found on the second to eleventh day, in addition to dengue virus antigens being detected in hepatocytes and capillary endothelial cells in the central lobular vein area.[10] When dengue virus was administered to mice, virus detection in serum, spleen, liver and brain indicated virus dissemination and virus replication in various tissues.[5,6] When dengue virus serotype 2 was intraperitoneally injected into BALB/c mice, viremia and liver damage

were found, which were marked by progressive increases in SGOT and SGPT. In addition, dengue virus antigen was found on hepatocyte cells, Kupffer cells, and hepatocyte cells, indicating the presence of viral replication in these cells. Liver damage in the form of injury to hepatocyte cells, steatosis, edema and necrosis. This liver damage can be seen histologically, liver lesions appeared within 2 days after infection and peaked on day 7 of infection.[8,9,14] In BALB/c mice infected with dengue through intraperitoneal injection of serotype 2, the level of viremia was very low and there were no clinical manifestations but there was injury to the liver in the form of increased SGOT and SGPT and the presence of viral antigens histopathologically.[8] Liver damage in BALB/c mice infected with dengue virus was also suspected due to the role of the immune response as evidenced by the increased expression of pro-inflammatory cytokines early in infection followed by with an anti-inflammatory response.[15] Another study with BALB/c mice injected with dengue virus serotype 4 intravenously showed histopathological changes similar to those in humans and dengue virus was detected in the liver, heart, lung, and serum.[16] In this study all BALB/c mice that had Intraperitoneal injection of dengue virus serotype 2 were detected NS1 dengue antigen on the mice serum on the second day after injection of the virus. The findings of this study support the theory of BALB/c mice as an experimental animal model for research on dengue virus infection.

Endothelial cell activation plays an important role in the pathogenesis of dengue infection which triggers an inflammatory response to infection. In a study conducted by Cardier et al, it was proven that in the acute phase there was activation of microvascular endothelial cells as indicated by an increase in ICAM-1. Endothelial cell activation is mediated by TNFα released by infected monocyte cells. This study also proved that dengue infection stimulates the apoptosis of microvascular endothelial cells. [17] Intercellular Adhesion Molecule-1 is part of the immunoglobulin consisting of transmembrane (ICAM-1) and soluble protein (sICAM-1), expressed by various cells such as leukocytes, hepatocyte cells and endothelial cells in response to inflammation. In a case control study conducted by Conroy, sICAM-1 levels were higher in dengue infection than healthy controls and higher in DHF and DSS than in dengue without complications. This study describes the role of inflammation and endothelial cell activation in the pathogenesis of dengue infection.[18] ICAM-1 levels in the serum of dengue patients are dynamic according to the course of dengue infection where the highest levels are found in the critical phase.[19]

The level of sICAM-1 as a sign of endothelial activation was higher in the group of BALB/c mice infected with dengue when compared to the BALB/c mice that were not infected with dengue. This finding is evidence that dengue virus infection increases endothelial cell activation. Endothelial cell activation plays a role in the pathogenesis of dengue infection and sICAM-1 is a marker of endothelial cell activation. Excessive endothelial cell activation causes an increase in plasma permeation through activation of NF-kB in addition that endothelial activation also causes an increase in the production of pro-inflammatory cytokines. Increased levels of sICAM-1 can be used as a marker to predict the progression of infection to a more severe disease.[20] MIF (Macrophage Migration Inhibitory factor) and TM (thrombomodulin) concentrations increase in plasma of dengue hemorrhagic fever patients and the potential effect of MIF on coagulation through TM and ICAM -1 in endothelial cells and monocyte cells. Severe dengue infection stimulates the expression of MIF which then stimulates endothelial cells and monocyte cells to express TM and ICAM-1.[21]

Activation of vascular endothelial cells in the group of mice infected with dengue virus was higher in mice without infection, this can be proven by the higher levels of sICAM-1 in BALB/c mice with dengue infection Endothelial cell activation plays an important role in the pathogenesis of dengue infection which triggers an inflammatory response. Endothelial cell activation is mediated by TNFα which is released by infected monocyte cells.[15] Excessive activation of endothelial cells through NF-kB activation, further increases the production of various cytokines which causes increased capillary permeability. Moreover, the dengue virus is also said to directly infect endothelial cells and cause apoptosis and endothelial cell dysfunction which contributes to plasma vascular leakage.[22] We need the next research to reduce endothelial activation in dengue viral infection.

CONCLUSIONS

BALB/c mice were proven to be infected with dengue virus by detecting NS1 dengue virus antigen in the serum. The level of sICAM-1 as a marker of endothelial activation in the group of dengue-infected BALB/c mice was significantly higher when compared to BALB/c mice that were not infected with dengue.

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