Activity Test of Dexamethasone Therapy to Humoral Immunity in Balb/c Mice with Biliary Atresia

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ABSTRACT

The prevalence of biliary atresia is 1:5,000-8,000 of live births. Kasai portoenterostomy is a reliable treatment, but liver damage continues. Dexamethasone (corticosteroid) evolved into a commonly used therapy and is believed to improve clinical outcomes in biliary atresia. The objective of this study was to determine the effect of dexamethasone on the absolute number of IgA, IgD, IgE, IgG, and IgM in mice Balb/c model of biliary atresia. Murine model of biliary atresia obtained by injection of 20 µL of PBS containing 10^6 pfu Rhesus rotavirus (RRV) at <24 hours of mice age. Injection of dexamethasone with dose of 0.5 mg/kg body weight was done on day 7 to day 14, and day 14 to day 21 after virus injection. Spleen taken for analysis of flow cytometry. Data were tested with Kruskal-Wallis test, then follow up the Man-Whitney test to assess the difference with SPSS 16.0 for Windows. Here, we show significant difference of immunoglobulin molecule in both termination (p<0.05). Subcutaneously injection of RRV (Rhesus Rotavirus) in Balb/c mice in the aged <24 hours is capable to stimulate the production of immunoglobulin, especially at the termination of the third week. This can be evidenced by an increase in IgD, IgA, IgD, and IgG. Dexamethasone is glucocorticoid that plays role as immunosuppressant with ability in decreasing IgD, IgM, IgD, and IgG levels in both termination. Furthermore we showed that dexamethasone was capable of stimulating the production of IgA in third week.

Keywords : Balb/c baby mice, Biliary atresia, Corticosteroid, Dexamethasone, Rhesus Rotavirus

INTRODUCTION

The evolution of the immune system in mammals have evolved as a defense mechanism against microorganisms that invade the body. This system is important in regulating the body's homeostasis. Regulatory T cells play a key role to maintenance normal homeostasis [1,2]. Biliary atresia is a progressive inflammatory obstruction of extrahepatic and intrahepatic bile ducts, which occurs in the first few months of a baby's life [3]. Prevalence of this disorder is 1 in 5000-8000 live births, and estimated that there are 400-500 new cases each year, as well as an indication of 50-60% of liver transplants in children [4].

Portoenterostomi by Kasai operation found in 1959 is a major breakthrough in the management of biliary atresia. The success of the Kasai operation was higher in patients operated on less than 2 months of age. Without surgery, the patient will usually die by age 2 years. Kasai operation is a reliable treatment, but liver damage continues even after Kasai surgery [5].

Dexamethasone is a synthetic glucocorticoid with immunosuppressant activity, choleretic effect and anti-inflammatory. As an immunosuppressant, dexamethasone works by lowering the immune response to stimulation [6].

B lymphocyte cells that have been transformed into plasma cells to produce antibodies that are neutralizing or opsonizing, the only effective way to eliminate the virus while outside the host cell. The nature intended neutralization of antibodies to neutralize the virus antigen has properties of a cell damaging (cytopathic effect), whereas opsonization function is so easy to clean the virus particles by phagocytic cells. It is known that phagocytosis is also supported by the complement system may
play a role in the break directly owned by the viral envelope [7]. Therefore, this study is important to determine whether corticosteroids can modulate the activity of immunoglobulin of Balb/c mice with biliary atresia.

METHODS

This research was conducted on July 2013 until January 2014 in Laboratory of Animal Physiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang. The animal experiments were approved by the Animal Care and Use Committee of the Brawijaya University.

Experimental Design. This study uses experimental animals Balb/c mice newborn. The sample selection of baby mice population and the placement of the group try, induction of rotavirus (R), dexamethasone therapy (RD) and control (K) the allocation is done randomly. This study consists of two phases. The first stage is a pre-condition where the bile ducts are made of baby mice induced fibrosis with 20 mL of phosphate buffered saline containing 1.5x10⁶ fluorescence-forming units Rhesus rotavirus (RRV) subcutaneously in the first 24 hours after birth. The second stage, baby mice were induced dexamethasone 0.5 mg/kg BW subcutaneously on days 7 and 14 after induction of RRV. Further investigated the therapeutic effect of dexamethasone on days 14 and 21 after induction. This study has received Ethical Clearance from the Research Ethics Committee (Animal Care and Use Committee) UB No. 391 / EC / IEC-S3 / 11/2012.

Virus Injection. Viruses that used as injection material is Rhesus rotavirus (MMU18006) American Type Culture Collection (ATCC®VR 1739TM). Preconditions to induced fibrosis with 20 mL of phosphate-buffered saline containing 1.5x10⁶ fluorescence-forming units Rhesus rotavirus (RRV) subcutaneously in the first 24 hours after birth.

Dexamethasone Therapy on Balb/c Mice with Biliary Atresia. Mice that had been infected with RRV then induced with 0.5 mg / kg body weight subcutaneously dexamethasone on day 7 and 14. Mice that had been injected with dexamethasone on day 7 to day 14 was terminated on day 14, and mice that had been injected with dexamethasone on day 14 to day 21 terminated on day 21.

Spleen Isolation. Spleen that had been isolated from mice was washed with PBS. After that, spleen was pushed clockwise using the base of the syringe and filtered with a wire. Homogenates were mixed with PBS included in propylene tube and add 15 ml of PBS until the volume reached 10 ml. Homogenates then were centrifuged at a 2500 rpm, 4°C for 5 minutes. Supernatant was discarded while the pellet was taken and resuspended with 1 ml of PBS and homogenized.

Flow Cytometry Analysis. Preparation with Cytofix / Cytoperm kit (BD-Biosciences PharMingen) and Washperm to performed intracellular staining. Pellets on microtube were added with 50 mL dye-specific antibodies. Type of coloring that had been used is triple staining with a combination of 3 types of antibodies specific cellular markers to be analyzed. Monoclonal antibody used is a combination of fluorescein isothiocyanate (FITC)- conjugated α-mouse B220 (klon: RA3-6A2/Biolegend), phycoerythrien (PE)- conjugated α-mouse IgE (klon: RME-1/Biolegend), phycoerythrien (PE)- conjugated α-mouse IgD (klon: 11-26c.2a/Biolegend), phycoerythrien-Cyanine dye 7 (PE-Cy7)- conjugated α-mouse IgM (klon: RMM-1/Biolegend), phycoerythrien-Cyanine dye 7 (PE-Cy7)- conjugated α-mouse IgA (klon: bs-0774R/BioSS), phycoerythrien-Cyanine dye 7 (PE-Cy7)- conjugated α-mouse IgG (klon: Poly 4053/Biolegend). The Samples run with BD FACS Calibur™ flowcytometer.

Data Analysis. This study was tested with the Kruskal-Wallis test using a significance level (α) of 0.05. The data used in the form of changes in the quantity of the absolute number of immunoglobulin. If the obtained p< 0.05, the results showed significance between each treatment. Furthermore, the follow-up Mann-Whitney test with α of 0.05 If p <0.05 then shows the real difference between the two
treatments were compared. Data analysis was performed using SPSS 16.0 for Windows.

RESULT AND DISCUSSION

The Absolute Number of IgD. The results of flow cytometry analysis of the spleen (Figure 1) showed that the number of IgD cells increased significantly in the R group (0.1 million of cells) compared to K group (0.04 million of cells) and decreased back to the RD (0.05 million of cells) in the first termination (second week). The results of the analysis of the number of IgD in the second termination (third week) showed that the cell number increased significantly in the R group (0.11 million of cells) compared to K group (0.04 million of cells) and decreased back to the RD (0.08 million of cells).

Figure 1. The absolute number of IgD (K = control, R = Administration of Rotavirus and RD = administration of Rotavirus and Dexamethasone).

Note: Different letters indicate significant difference by Mann-Whitney test

In vitro studies showed the effect of dexamethasone may increase the transcription of anti-inflammatory proteins, so it will evolve a Th naive to Th 2. T helper 2 secrete IL-4, IL-5, and IL-10 which affect B cells to form immunoglobulin [8]. In the second RD treatment termination showed decreased absolute number IgD. This is possible because the system is different.

The Absolute Number of IgA. The results of flow cytometry analysis of the spleen (Figure 2) showed that the number of IgA cells increased significantly in the R group (0.15 million of cells) compared to RD group (0.08 million of cells) and decreased back to the K (0.02 million of cells) in the first termination (second week). The results of the analysis of the number of IgA in the second termination (third week) showed that the cell number increased significantly in the RD group (0.13 million of cells) compared to R group (0.05 million of cells) and decreased back to the K (0.01 million of cells).

Figure 2. The absolute number of IgA (K = control, R = Administration of Rotavirus and RD = administration of Rotavirus and Dexamethasone).

Note: Different letters indicate significant difference by Mann-Whitney test

IgA molecules bind function envelop virus fusion with the plasma membrane of the target cell. In this activity in addition to IgA, IgM and IgG also plays a role. Secretory IgA inhibits viral binding to target cells, thereby preventing infection or reinfection [9].

The Absolute Number of IgG. The results of flow cytometry analysis of the spleen (Figure 3) showed that the number of IgG cells increased significantly in the R group (0.14 million of cells) compared to K group (0.09 million of cells) and decreased back to the RD (0.08 million of cells) in the first termination (second week). The results of the analysis of the number of IgG in the second termination (third week) showed that the cell number increased significantly in the R group (0.47 million of cells) compared to K group (0.14 million of cells) and decreased back to the RD (0.12 million of cells).
IgG has a function as part of a secondary immune response to an antigen, after the primary response by IgM. IgG can cross the placenta in mammals and is responsible for the protection of newborn infants in early life. IgG distribution in intra-and extravascular spread. IgG is able to be a partner to complement in eliminating an antigen [10].

The Absolute Number of IgM. The results of flow cytometry analysis of the spleen (Figure 4) showed that the number of IgM cells increased significantly in the R group (0.03 million of cells) compared to RD group (0.007 million of cells) and decreased back to the K (0.005 million of cells) in the first termination (second week). The results of the analysis of the number of IgM in the second termination (third week) showed that the cell number not significant between R group (0.05 million of cells) compared to K group (0.06 million of cells) and decreased back to the RD (0.06 million of cells).

IgM antibodies play a role in the initial response to an antigen which has never infect the body of an individual. IgM antibodies are produced in significant quantities in the first exposure to an antigen. IgM is able to activate the complement system and can cause cell phagocytosis on APC. In addition, IgM and complement may play a role very efficient in causing lysis of a foreign microorganism. Free IgM pentamer-shaped structure, the antibody can thus act as an agent for the agglutination of microorganisms for elimination from the body [11].

The Absolute Number of IgE. The results of flow cytometry analysis of the spleen (Figure 5) showed that the number of IgE cells increased significantly in the R group (0.08 million of cells) compared to K group (0.05 million of cells) and decreased back to the RD (0.05 million of cells) in the first termination (second week). The results of the analysis of the number of IgE in the second termination (third week) showed that the cell number increased significantly in the R group (0.35 million of cells) compared to RD group (0.12 million of cells) and decreased back to the K (0.07 million of cells).

Results of research conducted Reimerink stated that rotavirus infection in newborn babies do not have a significant effect on levels of IgE.
IgE increases with age and severity of rotavirus infection [12].

**CONCLUSION**

RRV (Rhesus Rotavirus) injection subcutaneously in Balb / c mice aged <24 hours proved capable of stimulating the production of immunoglobulin, especially at the termination of the third week. This can be evidenced by an increase in IgD, IgA, IgD, IgG. Dexamethasone as a glucocorticoid proved to regulate the activity of immunosupresant. This can be evidenced by an decrease in IgD, IgM, IgD, IgG both first and second termination. Dexamethasone capable to stimulating the production IgA in third week.

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**REFERENCES**


