Profile of T cells after dexamethasone treatment in BABL/c mice

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ABSTRACT

Regulation of homeostatic mechanisms and the development of T lymphocytes is influenced by several things, one of them is glucocorticoids. Dexamethasone is a synthetic glucocorticoid drugs used in the treatment for several diseases acted as an anti-inflammatory. The aim of this experiment was to confirm the effect of dexamethasone at normal dose and high dose on T cell profile and also to know the quantity of T cells after dexamethasone injection in each dose. This experiment we applied 18 mice in the age of 2 week and divided into 3 treatment groups with six replication i.e. control, dexamethasone injection with normal dose (0.5 mg/kg BW) and high dose (10 mg/kg BW), then observed on day-7 after injection. T cells were isolated from the spleen and analyzed by flow cytometry. Data analysis was confirmed with the ANOVA test followed by Tukey test with significance different (α) of 0.05. The result showed that dexamethasone act as immunosuppressant agent on high dose (10 mg/kgBB). Dexamethasone injection with normal dose on healthy mice showed no significant different in total number of CD4+, CD8+, CD4+CD62L, and CD8+CD62L compared to control. But, dexamethasone injections with high dose showed that the total number of CD4+, CD8+, CD4+CD62L, and CD8+CD62L were decreased significantly.

Key words: Dexamethasone, Flowcytometry, Glucocorticoid, Immunosuppressant, T lymphocyte.

INTRODUCTION

Glucocorticoids are hormones produced by the adrenal glands under the control of the hypothalamus and pituitary. Steroids produced by these glands will lead to some action in the body's systems, including in the process of regulation of carbohydrates, proteins, and fats. In addition to these functions, glucocorticoids also affect the immune system mechanisms, which have the ability as an immunosuppressant when there is inflammation and is widely used as a therapy for some diseases such as asthma, arthritis, and other inflammatory diseases [1].

Along with the need for treatment of several inflammatory diseases, then several manufacture developed the synthetic glucocorticoid. One of the glucocorticoid synthetic example is dexamethasone. Dexamethasone known as adrenocortical steroid synthetic and that believed have the same ability as internal glucocorticoid that naturally produced in the body in dealing various diseases, including inflammatory diseases through regulation of immunocompetent cells that play a role in the inflammatory process [2].

Immunocompetent cell that was important in inflammatory process is a T lymphocyte cells. The Treatment with the use of dexamethasone can affect homeostatic mechanisms and the development of T lymphocytes [3]. On T cells, glucocorticoids cause changes in the immune function of several mechanisms, which can induce apoptosis of T cells that can suppress the transcription of some T cells that produce cytokines and increases regulatory T cell subpopulations. Dexamethasone is indicated as replacement therapy may be used if there is secondary adrenal insufficiency arising from the secretion of corticotropin deficiency [2,4].

In this paper we confirm and report the status and the quantity of lymphocyte T cells on healthy mice with Dex induced. The result will show the effect of Dex administration on healthy mice and how the quantity of lymphocyte T cells on Dex induced-mice.

METHODS

This experiment was started on September 2014 until April 2015 in Laboratory of Animal Anatomy and Physiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang.
Research Design. Eighteen healthy BALB/c mice, aged between two weeks were used and divided into 3 treatment group with six replication i.e. control (no injection), dexamethasone injection with normal dose (0.5 mg/kg BW) and high dose (10 mg/kg BW). The treatment consisted of a single injection of Dex by intraperitoneal technique.

Isolation of lymphocytes from spleen
Isolation lymphocyte cells performed on day 7 after Dex injection. Spleen was taken from mice had been dissected and cleaned with phosphate-buffered saline (PBS). Spleen was placed in a petri dish containing PBS then crushed with syringe. The homogenate then moved into propylene tubes and added with PBS till 10 ml. Then centrifugated at 2500 rpm, 4°C for 5 minutes. Pellet were taken from the suspension then homogenize with 1 ml PBS.

Antibodies Staining and Flow Cytometry Analysis. Pellets on micro tube were added with 50mL dye-specific antibodies. Monoclonal antibody used is a combination of fluorescent of fluorescein isothiocyanate (FITC) anti mouse CD-4 (klon : GK 1.5/Biolegend), pychoerythrien (PE) Rat anti mouse CD-8a (klon : 53-6.7) and PE / Cy5 antimouse CD-62L (klon : MEL-14). The suspension were transferred into cuvette then samples were ready for analysis according to the parameters on flowcytometry FACS Calibur machine. Obtained result analyzed by BD Cell Quest Pro™. Anti-bodies were provided by Muhaimin Rifa’i (Chief of Laboratory of Animal Anatomy and Physiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang).

Statistical Analysis. Data from BD Cell Quest Pro™ software was analyzed using one-way ANOVA then followed with Tukey test with significance level (α) of 0.05. Analysis was performed using SPSS 16.0 for Win-dows.

RESULT AND DISCUSSION
Dex treatment that was given to healthy mice showed immunomodulatory activity as immunosuppressant at high dose (10 mg/kg BW), but at normal dose (0.5 mg/kg BW) the healthy mice lymphocyte cells, it is known there were no significant immunosuppressant activity. This result can be seen through the total number in relative cells number of CD4+, CD8+, CD4 CD62L+ and CD8+ CD62L+.

Analysis of Relative Number of CD4+ and CD8+ Cells using Flowcytometry. This data showed that the relative number of CD4+ T cells at control has a number of relatively as much as 6,31% and when treated with Dex in normal dose the number of relatively as much as 5,76% but at high dose is 4,01% (Figure 1). This result also showed that Dex treatment can changes the relative number percentage of CD4+ on both of normal dose and high dose. But, based on ANOVA test showed that at normal dose there were no significant different number when compared with control treatment (P<0,05), whereas at the high dose the data showed that there were a significant different total number of CD4+ T cells between this dose treatment and the control treatment.

![Figure 1](image1.png)

**Figure 1.** The relative number of CD4+ from spleen with 3 treatment (Control = without treatment; D1 = 0.5 mg/kg BW; D2 = 10 mg/kg BW. Different letters indicate significant difference by Tukey test.)

![Figure 2](image2.png)

**Figure 2.** The relative number of CD8+ from spleen with 3 treatment (Control = without treatment; D1 = 0.5 mg/kg BW; D2 = 10 mg/kg BW. Different letters indicate significant difference by Tukey test.)
letters indicate significant difference by Tukey test. The second figure (Figure 2), showed that the number of CD8+ T cells at each doses much as 3.08% (control), 3.38% (D1) and 1.35% (D2). It is known that the Dex treatment at normal dose at healthy mice did not have a significant different number of CD8+ T cells \( P<0.05 \), when compared with the control treatment. The total number of both CD4+ and CD8+ T cells at Dex treatment with normal dose indicates that Dex treatment did not affect the activity and total number of CD4+ and CD8+ T cells on healthy mice. This result can be assumed because the mice which used in this experiment was a normal cell or a healthy mice without any antigen infection and no disease detected, so the cells doesn’t produce high number of CD4+ and CD8+ [5]. So, this immunosuppressive effect of this Dex did not seen its function as well.

The functional role of a protein may differ in various form on a cell depending on their micro environmental contexts, that also occur on this CD4+ and CD8+ number at normal dose in healthy mice. This evidence suggest that glucocorticoid or Dex treatment work based on this environmental condition. Glucocorticoid, when it present at physiological concentrations and low concentrations can have an anti-apoptotic action of thymocyte [6]. So, it is support the result of Dex treatment of the CD4+ and CD8+ number at normal dose did not have any significant different number with control treatment.

Whereas, different with the normal dose, at high dose (10 mg/kg BW) showed the total of CD8+ relatively number were decreased if compared with control treatment. Based on the result of flowcytometry analysis followed with ANOVA test, it is known that the treatment of Dexamethasone at high dose show there were a significant different number of CD8+ T cells between high dose treatment and control treatment \( P<0.05 \).

The total number of both CD4+ and CD8+ on high dose showed that there were a significant different total number between the high dose treatment and the control treatment. Thus result assumed that Dex treatment can affect the activity and the total number of CD8+ at healthy mice. High decrease of total number of the CD4+ and CD8+ can affect the homeostatic of immune system.

Glucocorticoid synthetic now known as anti-inflammation and have an immunosuppressant activity through its apoptotic ability on lymphocyte T cells [7]. Dexamethasone have an ability to suppress immune action in the body then have the same ability to suppress cytokine at several inflammatory disease [2]. The therapeutic effects of dexamethasone including their inhibitory effect on T cells immunity. Treatment of dexamethasone affect T cells immunity through variety of mechanism: Induce T cell apoptosis, inhibit the transcription of T cells to produce cytokine and enhances Treg population [4,7,9].

Treatment with dexamethasone can induced apoptotic mechanism by its regulation on transcription several genes and protein synthesis. There were 25 genes that participated in apoptotic mechanism of dexamethasone [3]. Glucocorticoid signaling increases the expression of the pro-apoptotic genes family, one of them is Bcl-2 family member Bim, which can activate the pro-apoptotic protein such as Bax and Bak that resulting in the release of cytochrome c and other apoptogenic proteins, then followed by caspase 9 and caspase 3 activation that leads apoptosis. Dexamethasone as glucocorticoid synthetic have a role to balance both of the pro and anti-apoptotic proteins [10]. So, from this explanation, it is assumed that one of the factor that can decrease number of CD4+ and CD8+ T cells at high dose in healthy mice because the ability of dexamethasone to induce apoptosis in lymphocyte T cells.

**Analysis of Relative Number of CD4+CD62L+ and CD8+CD62L+ Cells using Flowcytometry.** CD62L is a glycoprotein trans membrane that bind with carbohydrate group in high endothelial venules (HEV) on peripheral lymph nodes. CD62L have an ability as a key regulator for lymphocyte T cells migration then have an ability to control and modulate lymphocyte T cells to enter into lymph nodes after acute infection [11]. T cells that activated by antigen or infection will lose their CD62L expression in their cell surface [12].

The result of flow cytometry analysis of the spleen showed that the number of CD4+CD62L+ was not affected significantly after injection of dexamethasone with normal dose. That result had been shown with the
number of CD4⁺CD62L⁺ that different at each dose, the number of control treatment as much as 51.9%, normal dose treatment as much as 59.2% and then at high dose treatment there were 22.0% (Figure 3). This result also supported by ANOVA test that showed that there were no significant different (P<0.05) between the number of CD4⁺CD62L⁺ at normal dose treatment with control treatment (no injection). Same result also showed in the number of CD8⁺CD62L⁺ at the normal dose treatment (Figure 4). The flow cytometry result showed that there were no significant different between the number of CD8⁺CD62L⁺ at the normal dose with the number of CD8⁺CD62L⁺ at high dose. This result indicate that the dexamethasone treatment was not affected the activity and the number of both CD4⁺CD62L⁺ and CD8⁺CD62L⁺ from the total number of CD4⁺ and CD8⁺ T cells.

In the high dose with dexamethasone treatment, both CD4⁺CD62L⁺ and CD8⁺CD62L⁺ showed significant different number if compared with control treatment on healthy mice (P<0.05). This result indicated that in high dose dexamethasone can affect the activity and the number T cells, it is known by the decrease of number both of CD4⁺CD62L⁺ and CD8⁺CD62L⁺ T cell after high dose of dexamethasone injection.

L-selectin is a homing receptor for lymphocyte, and it is known that if the number of this molecule is low it can affect the number of T cell naïve, which is can decrease the number of T cell naïve and it can lead the homeostasis in the immune system unstable. It is known that activation of T cell decided by the existence of CD62L molecule in the surface of T cells. Activated T cell will lose this adhesion molecule and express CD44 molecule [13].

Expression of CD62L on T cell can regulated by several condition, CD62L will decrease its number by down regulated process in chronic inflammation condition [14, 15]. From this explanation it is assumed that the decrease number of CD4⁺CD62L⁺ and CD8⁺CD62L⁺ T cell have a worst effect to activation T cell on healthy mice. The decrease of this molecule adhesion after high dose dexamethasone injection caused by the ability of glucocorticoid to suppress adhesion of L-selectin and its interaction with integrin and endothelial. Glucocorticoid also have a down-regulated mechanism to adhesion molecule, L-selectin and β₂-integrin in the surface of the cell [16].

CONCLUSION

Dexamethasone treatment that was given to healthy mice showed immunomodulatory activity as immunosuppressant. But this activity clearly showed at dexamethasone treatment with high dose (0.05 mg/kg BW). Dexamethasone treatment with normal dose (0.5 mg/kg BW) showed that there were no significant different number between this dose with control treatment. Whereas, at high dose showed a high immunosuppressive activity that caused by significant lowering number of T cells lymphocyte including CD4⁺, CD8⁺, CD4⁺CD62L⁺, CD8⁺CD62L⁺.

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REFERENCES


