Expression of Erythroid Progenitor Cells and Erythrocytes on Dexamethasone Induced-Mice

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ABSTRACT

Dexamethasone (Dex) is synthetic corticosteroid, known as anti-inflamation drug to ameliorate autoimmune diseases. It worked by inhibiting production of proinflamatory citokines. The aim of this experiment was to confirm the effect of administration of Dex on erythroid progenitor cells, TER-119$^+$VLA-4$^+$ and erythrocytes, TER-119$^+$VLA-4$^-$ expression from bone marrow compartments. Two weeks old BALB/c mice were used and grouped into 3 injection treatments of Dex with six replications i.e. no injection (control), 0.5 mg/kg BW (dose 1), 10 mg/kg BW (dose 2). To investigate the therapeutic effect of dexamethasone, the mice were sacrified on day-7. The bone marrow cells were isolated and analyzed by flow cytometry. Data analysis was confirmed with the Kruskal-Wallis test followed by Mann-Whitney test with significance level (α) of 0.05. The result showed that administration of Dex on BALB/c mice increase the expression of erythroid progenitor cells, TER-119$^+$ VLA-4$^+$ and erythrocytes, TER-119$^+$VLA-4$^-$ from bone marrow.

Key words: Dex, erythrocytes, erythroid progenitor cells

INTRODUCTION

Dexamethasone (Dex) known as anti-inflamation drug, it was used for long time to ameliorate autoimmune diseases. Dex is synthetic corticosteroid that has similar mechanism with other corticosteroid hormones to inhibit production of proinflamatory citokines [1]. Past research showed that Dex has immunosuppressive activities, it lead TNFα and IL-6 decreasing when administrated by Dex [2]. But in other research report Dex has immunostimulant effect to up-regulate such complement, cytokine, chemokine receptors [3], stimulate blood cells differentiation, regulate mobilitation of blood cells and hematopoiesis on bone marrow [4,5].

Dex early mechanisms as potent regulators of various aspects of immunity begin when Dex and glucocortocoid receptor (GR) binds becoming Dex-GR complex. Dex-GR complex binds to glucocorticoid response elements in DNA level and modulate gene transcription. Dex-GR complex also possible to binds with transcription factor like activator protein-1 (AP1) and nuclear factor kappa B (NFκB) to inhibit and repress gene transcription [6-9].

Erythrocytes has significant role on body regulation and homeostasis. Erythrocytes produced on bone marrow, it develop from hematopoietic stem cells (HSCs). The growth and development of red blood cells lineage stimulated by growth factor from colony-stimulating factor (CSF) family [5].

In this paper we report the expression of erythroid progenitor cells and erythrocytes on Dex induced-mice. This result indicates that administration of Dex enhances the expression of erythroid progenitor cells, TER-119$^+$VLA-4$^+$ and erythrocytes, TER-119$^+$VLA-4$^-$ from bone marrow.

METHODS

This experiment was conducted on September 2014 until March 2015 in Laboratory of Animal Anatomy and Physiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang.

Research Design. Two weeks old BALB/c mice were used and maintained as experimental animals in animal facility. There are 3 injection treatment groups of Dex with six replications i.e. no injection (control), 0.5 mg/kg BW (dose 1), 10 mg/kg BW (dose 2). The injection was performed by intraperitoneal technique. Further investigate the therapeutic effect of Dex on day-7 after injection.

Bone Marrow Isolation. Whole mice was terminated and dissected to collect femur and tibia. Those organs were washed in PBS solu-

tion and flushed them by shyrange with needle into matrix to issolate bone marrow. Dilute squeezed-bone marrow into 15 ml poly-propylene tube with PBS until 10 ml. The suspension was centrifuged at 2500 rpm and 10°C during 5 minutes. Supernatant was discarded and homogenize 1 ml of PBS resuspended pellet.

Flow Cytometry Analysis. Analytical and data analysis of flow cytometry was performed by using FACS Calibur™ flow cytometer (BD-Biosciences, San Jose, CA) and BD-CellQuest Pro™ software. Monoclonal antibodies used for experiment were Biotin-conjugated anti-mouse TER-119 (clone TER-119) purchased from eBioscience Inc. San Diego, CA and pychoerythrien (PE)-conjugated anti–mouse VLA-4 (clone 9C10) purchased from BD Biosciences. Streptavidin-PE-Cy5 (eBioscience, San Diego, CA) was used to visualize biotin-conjugated antibody. 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 analysis was confirmed with the Kruskal-Wallis test followed by Mann-Whitney test with significance level (α) of 0.05. The data used in the form of changes in the quantity of the absolute number of erythroid progenitor cells, TER-119$^+$VLA-4$^+$ and erythrocytes, TER-119$^+$VLA-4$^-$. Analysis was performed using SPSS 16.0 for Windows.

RESULT AND DISCUSSION

Hematopoiesis is a development process of blood cells. It is very important to keep body homeostasis. Saveral studies report that hematopoiesis affected by extern factors, most of them are chemical compounds [10]. In this work, authors foccussing on extern factor that affected expression of erythroid progenitor cells and erythrocytes by administration of Dex with dose 0.5 mg/kg BW and 10 mg/kgBW on BALB/c mice animal model (Figure 1 and Figure 2).

Erythroid progenitor cells and erythrocytes are red blood cells lineage. Generally it from HSCs that produced by bone marrow. Stimulated by growth factors, HSCs will grow and develop becoming multipotent progenitors (MPPs) then follow its fate becoming two group of cells i.e. lymphoid cells and myeloid cells. Both erythroid progenitor cells and erythrocytes are myeloid cells [11,12] then chronologically it develop becoming colony forming unit granulocyte - erythrocyte - monocyte - megakaryocyte (CFU -GEMM), burst forming unit - erythrocyte (BFU-E), colony forming unit - erythrocyte (CFU-E) and mature erythrocytes [13].

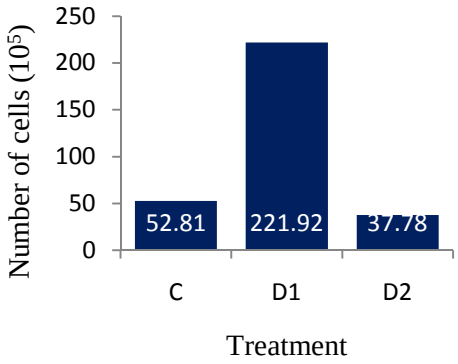

Figure 1. The absolute number of erythroid progenitor cells, TER-119$^+$VLA-4$^+$ from bone marrow after administrated by Dex. Control (C) without treatment; Dose 1 (D1) with 0.5 mg/kg BW; Dose 2 (D2) with 10 mg/kg BW. Different letters indicate significant difference by Mann -Whitney test.

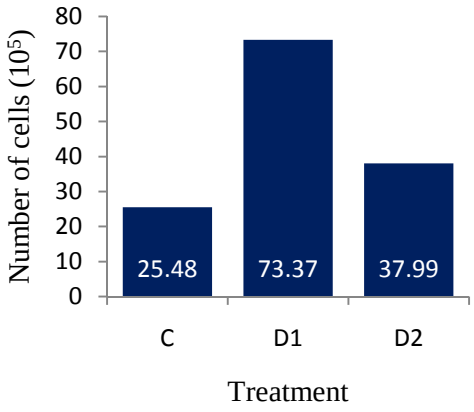

Figure 2. The absolute number of erythrocytes, TER-119$^+$VLA-4$^-$ from bone marrow after administrated by Dex. Control (C) without treatment; Dose 1 (D1) with 0.5 mg/kg BW; Dose 2 (D2) with 10 mg/kg BW. Different letters indicate significant difference by Mann-Whitney test.

Both erythroid progenitor cells, TER-119^{+}VLA-4^{+} and erythrocytes, TER-119^{+}VLA-4^{-} showed significant absolute number on treatment group by Dex with dose 0.5 mg/kg BW. One the other hand treatment group by Dex with dose 10 mg/kg BW showed insignificant absolute number, it similar with no treatment group (Figure 1 and Figure 2). It is mean Administration of Dex with dose 0.5 mg/kg BW more effective than other. High doses of Dex probably enchances apoptotic rate on immunocompetent cells. Signaling of Dex stimulate pro-apoptotic gene i.e Bim, then it activate pro-apoptotic protein to lead apoptosis on the immunocompetent cells i.e. B cells [14] and CD4^{+}CD25^{-} T cells [15].

Administration of Dex enhances the number of CD4^{+}CD25^{+} regulatory T cells [15] and it was revealed that CD4^{+}CD25^{+} regulatory T cells stimulate the development stage of TER-119 cells [16]. Based on Konto-Ghiorghi, glucocorticoid have been up-regulate CFU-E and increase erythrocytes proliferation dispite of erythropoietin (Epo) limitation [17]. Another research report that Dex enhances differentiation on BFU-E level as early stage of erythroid progenitor formation. Based on Narla et al., Dex increased the expression of suppressor of cytokine signaling-1 (SOCS-1) which has function in control the number of hematocrit and maturation process of red blood cells. Dex also increase the expression of Janus kinase 2 (JAK2) which play significant role in erythropoiesis [18].

The molecular mechanism of Dex toward erythropoiesis still unclear, but in some investigations reveal that Dex up-regulate saveral erythropoiesis-spesific genes like GATA-1, Flk-1 and Epo-R. The up-regulated level of GATA-1 would stimulate erythroid differentiation. It is known that glucocorticoid cooperates with Epo-R can induce long-term proliferation of erythroid progenitors [19]. By these experiment it is clear that Dex enhances the expression of erythroid progenitor cells, TER-119^{+}VLA-4^{+} and erythrocytes, TER-119^{+}VLA-4^{-}.

CONCLUSION

Administration of Dex with dose 0.5 mg/kg BW significantly increase the expression of erythroid progenitor cells, TER-119^{+} VLA-4^{+} and erythrocytes, TER-119^{+}VLA-4^{-} on BALB/c mice.

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