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The Lymphocytes Number in the Blood of Kwashiorkor Rat Model Induced by Oral Immunization with 38-kDa *Mycobacterium tuberculosis* Protein

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Abstract**—**Kwashiorkor is one of nutritional problem in Indonesia, which lead to decrease immune system. This condition causes susceptibility to infectious disease, especially tuberculosis. Development of new tuberculosis vaccine will be an important strategy to eliminate tuberculosis in kwashiorkor. Previous research showed that 38-kDa *Mycobacterium tuberculosis* protein is one of the potent immunogen. However, the role of oral immunization with 38-kDa *Mycobacterium tuberculosis* protein to the number of lymphocytes in the rat model of kwashiorkor is still unknown. We used kwashiorkor rat model groups with 4% and 2% low protein diet. Oral immunization with 38-kDa *Mycobacterium tuberculosis* protein given with 2 booster every week. The lymphocytes number were measured by flowcytometry. There was no significant difference between the number of lymphocytes in the normal rat group and the kwashiorkor rat groups. It may reveal the role of 38-kDa *Mycobacterium tuberculosis* protein as a potent immunogen that can increase the lymphocytes number from kwashiorkor rat model same as normal rat.

Keywords**—**kwashiorkor rat, lymphocytes, 38-kDa Mycobacterium tuberculosis protein

I. Introduction

KWASHIORKOR is one of the nutritional problems in Indonesia, caused by low dietary intake of protein.[1] It is a type of malnutrition which happens mostly on children, eventhough it could also happen on adult person. It is caused by protein deficiency combined with energy and micro nutrient essence deficiency. Decline in nutritional status is related with the loss of protection to infectious diseases, caused by decreased humoral and cellular immunity. So that kwashiorkor persons susceptible to infectious diseases, one of which is tuberculosis.[2]-[4]

Tuberculosis is a major health problems in Indonesia. Indonesia is the third contributors of tuberculosis cases in the world, after India and China. Mortality which caused by tuberculosis in Indonesia is approximately 140.000 per years.[5] Therefore, the data shows the need of ways to take care of tuberculosis maximally, such as by finding the case immediately, appropriate medical treatment, and vaccination.

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Malnutrition is an important risk factor for tuberculosis (TB) because cell-mediated immunity (CMI) is the key host defense against TB.[2],[6] The experimental evidence suggests that malnutrition can lead to secondary immunodeficiency that increases the host's susceptibility to infections.[7]

The recent finding showed that the development of vaccine based on the adhesin of bacteria molecule is effective. Adhesin bacterial which combined with potent adjuvant mucosal as mucosal delivery system, is a promising approach for mucosal vaccine.[8]-[10]

The previous research gained result that 38-kDa *Mycobacterium tuberculosis* protein was an adhesin protein on mouse enterosit and administration of it orally can induce CD4+ T lymphocyte and CD8+ T lymphocyte in intestine and lung of mice.[11],[12] Whether on kwashiorkor rat, the effect of 38-kDA *Mycobacterium tuberculosis* protein toward the lymphocyte count in the blood as cellular immunity is not found yet.

II. Material and Methods

A. Experimental Design

This research is a true experimental laboratory using rat as an animal model. Rats were divided in 3 groups: normal rat group with normal diet, and kwashiorkor rat groups which is given with 2% and 4% protein diet. Each of rat group is treated by 38-kDa *M. tuberculosis* adhesin protein with concentration 100µL/100gr body weight orally for 3 weeks.

B. Kwashiorkor Rat Model

Rattus novergicus strain wistar male, age 4 weeks is given with low protein diet 2% and 4%, for 6 weeks. The food composition of low protein diet refers to Kaladhar et al.[13] with modification. Whether the food composition as follows:

1.Standard (normal) diet

Normal diet is a confeed-pars, consist of protein 11%, fat 4%, fiber 7%, calsium 1,1%, phospor, coccidiostat 0,9%, and wheat flour 33,4%.

2. The 2% protein diet from total calorie

Composition of this food are: sugar cane, wheat flour, vegetable oil. The composition of nutrient are carbohydrates 50%, protein 2% and fat 31%.

3. The 4% protein diet from total calorie

Composition of this food are: sugar cane, wheat flour, vegetable oil. The nutrient composition are carbohydrates 50%, protein 4% and fat 29%.

C. Mycobacterium tuberculosis culture method

Method of bacteria culture is refer to Shah et al. [14] Bacteria culture is using *Lowensteen Jensen* (LJ) medium. LJ medium made on reaction tube size 10 m, incubated on 37° temperature for 4 to 8 weeks. The bacteria were taken when it showed a specific colonial growth.

D. Electrophoresis of Mycobacterium tuberculosis cell walls

The 38-kDa protein of *M. tuberculosis* is determined using SDS-PAGE [15] with modification. Sample of protein heated 100° for 5 minutes in propping liquid which contain Tris HCl 5 mM pH 6,8, 2-mercapto etanol 5%, sodium dodecyl sulfate 2,5% w/v, glycerol 10% v/v, Bromopherol blue color tracker. The chosen separating gel concentration was 12,5% mini slab gel with stacking gel 3%.[

E. Rats immunization with 38-kDa Mycobacterium tuberculosis protein

Preparation was carried out by modification from Harlow and Lane.[16] The 38-kDa *M. tuberculosis* protein with concentration 20 mg per 3 mL PBS mixed by Iscom 0,500 mg/mL PBS. Then glutaraldehyde 2% in 6 mL PBS was added, incubated in room temperature for 1 hour while steered slowly. Then 200 mM glycine pH 7,2 was added and incubated in room temperature for 1 hour, steered slowly. After that it was dialyzed using PBS 4 times in one night. Keep in form of aliquot and keep on temperature -20°C. Result of conjugation of 38-kDa *M. tuberculosis* adhesin protein with ajuvan Iscom is ready to use as rat immunization.

F. Lymphocyte Analysis

The number of lymphocytes in the blood of rats was calculated by flowcytometry method using blood analyzer Micros 6.0.

G. Statistical Analysis

Analysis of Variance test is carried out to find out the effect of 38-kDa *M. tuberculosis* adhesin protein giving toward the number of lymphocytes in the blood on various group of rats.

III. Result

The results on rats body weights performance after given with normal diet and low protein diet for six weeks are shown in Fig. 1. It shows that the body weight of rats groups with low protein diet were significantly ($p < 0.05$) lower compare to rats group with normal diet.

Table I shows the number of lymphocyte on the blood of rats groups. The number of lymphocyte on rats group with low protein diet were not significantly different ($p > 0.05$), from the normal rats group.

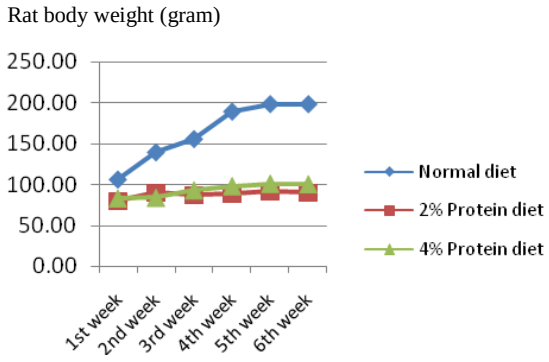

Fig. 1 Body weight of the rats

TABLE 1
THE LYMPHOCYTE NUMBER ON THE BLOOD

Group of Rats with diet	The number of Lymphocyte ($x10^3/mm^3$) Mean±SD
Normal Diet	4,04 ± 1,06
4% Protein Diet	3,58 ± 0,71
2% Protein Diet	2,94 ± 0,59

IV. Discussion

Kwashiorkor is a form of protein-energy malnutrition. The kwashiorkor–induced group of experimental animals by giving low protein diet was characterized by retarded growth, dermatitis, oedema, hair loss, physical inactivity, observable loss of motor co-ordination and apathy. [17] In this research, the rats which is given low protein diet show a retarded growth of body weight. The rats with low protein diet (2% protein diet and 4% protein diet) also have oedema, hair loss and physical inactivity (the images of the rats are not shown).

Kwashiorkor has a profound effect on cellular immune function. Many of the infections seen in patients with malnutrition, one of which is tuberculosis. Malnutrition is an important risk factor for tuberculosis, because cell-mediated immunity is the key host defense against TB.[7] So, the findings of effective immunization for the kwashiorkor person is important.

This research explored the ability of 38-kDa *M. tuberculosis* protein to induce the increase number of lymphocyte on the blood of kwashiorkor rat model. Based on these results, it can be seen that the number of lymphocytes in the normal diet group was higher than all the low-protein diet group, but there is not significantly different. Thus, the immunization of kwashirokor rats with 38-kDa *M. tuberculosis* protein orally can induce the enhancement lymphocytes number on the blood as well as normal rats. The 2% protein diet group had the lowest number of lymphocytes when compared with other groups. The reason for this finding is the protein plays an important role in the maturation of the immune system.[18] Thus, when the amount of protein consumed is less than demand, leads to a reduction on cell mediated immunity, and consequently the number of lymphocytes is also reduced. Decline in the number of lymphocytes is also associated with a lower intake of the amino acid glutamine in the diet. Kew[19] states that the lymphocytes require glutamine, both in resting and activated conditions by mitogen.

Glutamine is important for lymphocyte function, as well as an energy source and nucleotide precursor. In the catabolic state of the injury and illness, the amino acid glutamine becomes conditionally-essential, so requiring additional food or supplements. Glutamine deficiency has a negative effect on the functional integrity of the gut and cause immunosupression. Inadequate glutamine on the diet could inhibit lymphocyte proliferation, which ultimately affects the number of lymphocytes in the circulation. Glutamine is found in all protein-containing foods, with varying amounts. However, high levels of glutamine are found in wheat, which is the material for flour. This is consistent with the composition of the flour in a food mixture of rats as a dietary protein on low-protein diet or normal diet.[20]

Suppression of cell mediated immunity is something that is typically found in almost all patients with protein energy malnutrition. This is because of the formation of various immune system in the body depends on the body's ability to synthesize protein.

The previous research showed that 38-kDa *M. tuberculosis* protein was an adhesin protein on mouse enterosit and administration of it orally can induce CD4+ T lymphocyte and CD8+ T lymphocyte in intestine and lung of mice.[11],[12] In this research, the lymphocyte number on the blood of kwashiorkor rats is as much as normal rats, after immunization with 38-kDa *M. tuberculosis* protein orally. Thus, it can be said that despite inadequate intake of protein is given on the rats, the 38-kDa adhesin protein of *M. tuberculosis* could enhance the cellular immune system. But the adequate protein supplement will increase the maturation of cellular immune system optimally.[21] Further studies are also required to examine the effect of oral immunization with 38-kDa *M. tuberculosis* protein on humoral immune response in kwashiorkor rats.

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