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.

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 norvegicus 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%, calcium 1.1%, phosphor, coccidiostat 0.9%, and wheat flour 33.4%.

2. The 2% protein diet from total caloric

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 caloric

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 Lovensteen 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
10° 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, Bromophorle 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 flowcometry 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.

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
kwashiorkor 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 immunosuppression. 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 supplement will increase the maturation of cellular immune system. But the adequate protein will increase the maturation of cellular immune system.

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