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THE ROLE OF POLYSACCHARIDE KRESTIN FROM *Coriolus versicolor* MUSHROOM ON IMMUNOGLOBULIN ISOTYPE OF MICE WHICH INFECTED BY *Mycobacterium tuberculosis*

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ABSTRACT

This research was aimed to determine the role of polysaccharide krestin (PSK) with different timing on levels and types of mice immunoglobulin (Ig) isotype which infected by Mycobacterium tuberculosis. This research used 30 adult female mice of Mus musculus strain, polysaccharide krestin was isolated from Coriolus versicolor mushroom, and for infection used Mycobacterium tuberculosis H37Rv (ATCC 27294 T) strain. Provision of polysaccharide krestin was done over 7 consecutive days via gavage. Mycobacterium tuberculosis infection was done 2 times with an interval of 1 week via intraperitoneal. Immunoglobulin isotype serums were analyzed using the ELISA test and the results were analyzed descriptively through the color reaction and OD values. The result showed the highest levels of immunoglobulin was found in the provision of PSK before and after Mycobacterium tuberculosis infection with total 6.280 of OD Ig isotype. Immunoglobulin isotype dominant was IgM with lambda light chain. The conclusion of this research was PSK increased mice Ig isotype levels at the time of provision before, after or before and after infection Mycobacterium tuberculosis. Ig isotype which was formed i.e. IgM, IgA, IgG2b, IgG3, IgG2a, IgG1 with kappa and lambda light chain.

Key words: *Polysaccharide krestin, Mycobacterium tuberculosis, immunoglobulin isotype*

INTRODUCTION

Tuberculosis (TB) is still become a serious problem in the world [12].This bacteria is divided into extracellular and intracellular bacteria[2]. Specific response against extracellular bacteria with produce antibodies by B cells. While in response against intracellular bacteria, the response that happens is the cellular immune response (T cell) [7]. However, intracellular bacteria can induce the development of T cells into Th1 cell phenotype then also can stimulate antibody production by B cells [5].

In the early formation of immunoglobulin molecules (antibodies) by B cells is stimulated by antigen [9]. In mice, the class of immunoglobulin (Ig) based on the H-chain (heavy chain) consists of IgM, IgG, IgA, IgD, and IgE. In mice, IgG consists of four subclasses i.e. IgG1, IgG2a, IgG2b, and IgG3 [23]. In addition, there are 2 types of L-chain (light chain), namely kappa (κ) and lambda (λ) [19].

Some researchers use the immunomodulator as an adjunctive therapy for tuberculosis [18]. *Coriolus versicolor* is a mushroom that commonly used in the treatment of disease. Various active components are isolated from this mushroom, both taking from fruiting bodies or culture mycelium. Active components that are important in the treatment are polysaccharide krestin (PSK) and polysaccharide peptide (PSP). Both PSK and PSP consist of active compounds named β-glucan [16]. Beta (β)-glucan plays a role to activate macrophages and stimulate B cells in the process of antibodies production [3]. Beta glucan increase the production of important *cytokines* there is interleukin-2 (IL-2) which stimulates the differentiation of B cells which are active [29] then the active B cell differentiation into plasma cells (clones plasma) which can produce immunoglobulin [30].

Looking at the capabilities of the PSK on the modulation of immune responses and saw its consumed in a long time in the community without significant side effects, the researcher wanted to investigate how the levels and kinds of immunoglobulin isotype of mice which infected by *Mycobacterium tuberculosis* on providing PSK with

different timing. *Enzyme-linked immunosorbent assay* (ELISA) became selected test for measuring the levels and kinds of immunoglobulin isotype related to the specificity of antigen[7].

METHOD

Stage in PSK isolation from *Coriolus versicolor*

Coarse powder of 200 g *Coriolus versicolor* is added with water as much as 3 l and is heated at a temperature of 80–98° C for 2 - 3 hours. Do extraction twice more with the addition of 2 l of water on the residue, the results obtained in form of supernatant from the three times extractions are ± 2 l [10]. Mushroom extract solution is filtered using Whatman No 41 filter and then its *liofilisasi* supernatant (for 150 ml for ± 24 hours). Precipitation mushroom powder extracts using ammonium sulfate 90% and then dialysis using nitrocellulose membranes for 24 hours [11].

Stage of making PSK solution

3.5 g of Ammonium sulfate is added with 50 ml aquades and 1 g of mushroom powder mixed into one. Stirer solution for 2 h at 4° C and then centrifuged 9000 rpm for 12 min at 4° C. Take the sediment and added 12 ml saline. Polysaccharide concentration is measured using the *phenol-sulfuric acid assay*. Dose of PSK that used is 500 µg[29].

Stage of provisioning PSK and *Mycobacterium tuberculosis* infection

Thirty animals are divided become 6 groups as follows

Table 1. Treatment Group

Group	Provision of PSK on 1st-7th day	*Mycobacterium tuberculosis* infection on 8th and 15th day	Provision of PSK on 23th-30th day
I	–	–	–
II	+	–	+
III	–	+	–
IV	+	+	–
V	–	+	+
VI	+	+	+

Description: (+) indicates treatment
(-) indicates no treatment, were given only aquades
I : As a control, have given only aquades
II : As a positive control, provision of PSK only
III: As a negative control, Mycobacterium tuberculosis infection only
IV : Provision of PSK before infection with Mycobacterium tuberculosis
V : Provision of PSK after infection with Mycobacterium tuberculosis
VI: Provision of PSK before and after infection with Mycobacterium tuberculosis
Mice infected with 0.5 Mc Farland or equivalent to 1.5 ×108 CFU/ml bacteria intraperitoneally.

Stage of analysis Ig isotype

Serum Ig isotype (IgG1, IgG2a, IgG2b, IgG3, IgA, Ig M, kappa and lambda chains) were analyzed with Pierce Rapid ELISA Kit Mouse Mab Isotyping. The reading of OD values by using ELISA reader at a wavelength of 450 nm[4].

RESULT

Table 2. OD Values of Ig Isotype

Kinds of Ig	*Optical Density* (OD) values of Ig					
	K	(K+)	(K-)	P1	P2	P3
IgG1	0,247	0,393	0,304	0,652	0,479	0,755
IgG2a	0,338	0,400	0,426	0,697	0,661	0,872
IgG2b	0,705	0,972	0,908	1,029	0,986	1,041
IgG3	0,414	0,780	0,573	1,000	0,654	1,047
IgA	0,322	0,968	0,953	1,108	1,015	1,155
IgM	0,909	1,286	1,349	1,335	1,420	1,410
Kappa	0,438	0,909	0,806	1,119	0,945	1,077
Lambda	0,584	1,068	1,100	1,112	1,180	1,169

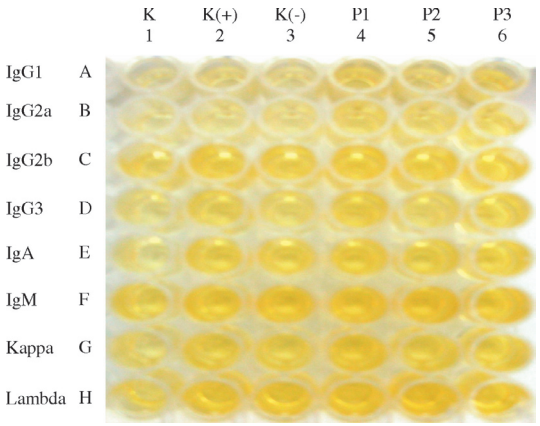

Figure 1. ELISA test for determining the kinds and levels of immunoglobulin Description: K (1A-1H) control, K (+) (2A-2H) positive control is provision of PSK only, K (-) (3A-3H) negative control with *Mycobacterium tuberculosis* infection only, P1 (4A-4H) provision of PSK before infection with *Mycobacterium tuberculosis,* P2 (5A-5H) providing PSK after infection with *Mycobacterium tuberculosis,* P3 (6A-6H) providing PSK before and after infection with *Mycobacterium tuberculosis*.

DISCUSSION

In serum (K) Although there has been color reaction, but not so striking as in serum (K+). This is because the control (K) not be immunized previously with antigens, which meant there was no previous contact with antigens. Color reaction that occurred probably due to the presence of natural antibodies in the body of mice whose concentration is low.

In (K+) provision of PSK only, it has OD values higher than (K). High concentration of immunoglobulin appropriate with the statement of Bellanti (1993), that the potential immunomodulator can increase or make higher levels of certain responses as a whole. According Vetvicka *et al.* (2002), Beta-glucan is known to increase the production of lymphocytes.

In (K-) OD value is higher than (K). This is because *Mycobaterium tuberculosis* can not make invasion of the immune system so it does not decrease the immune response. According Todar (2009), *Mycobaterium tuberculosis* can be multiply after 7–21 days early after infection and Abbas *et al.* (2000) states that the maximum antibody in the primary response can be detected in the third week after immunization. Kresno (2001) states that levels of antibody reduced later and generally only a few can be detected on 4–5 weeks after exposure.

Tuberculosis bacterial population are divided into extracellular and intracellular bacteria[2]. Immunoglobulin which produced by B cells is the major protective immune component for extracellular bacteria that can serves to get rid of microbes and neutralize the toxin [5]. In the fight against intracellular bacteria there are 2 types of reaction are occurred, i.e. The first is killing of intracellular bacteria by macrophages activated through phagocytes in which the activation of macrophages occurs through cytokines, especially IFN-γ, produced by T cells. The second way is with lysis of infected cells by $CD8^+$ T cells. Intracellular bacterial protein can stimulate $CD4^+$ T cells (through MHC class II antigens complex) or $CD8^+$ T cells (through MHC class I antigens complex). Intracellular bacteria induce T cell development into Th1 cell phenotype, because these bacteria stimulates the production of IL-12 by macrophages, and IFN-γ by NK cells, both types of these cytokines promote the development of Th1 cells ($CD4^+$). On the other hand Th1 cells produce IFN-γ which activate macrophages to produce ROI and enzymes that can kill bacteria. IFN-γ also stimulate immunoglobulin isotype production by B lymphocytes [19].

In (K-) has a lower OD value than the P1, P2 and P3. This shows PSK has a role as immunostimulator. This is consistent with the statement of Cui and Chisti (2003), Kidd (2000), and Vetvicka *et al.* (2002), that the PSK is immunostimulator or imunopotensiator.

Polysaccharide krestin contains 34–35% carbohydrate (91–93% glucan) [10]. Beta (β)-glucan is known for stimulate the immune system [24], According to Hong *et al.* (2004), Beta (β)-glucan present in the gut then make contact with macrophages that exist in the intestinal wall which is assisted by M cells (microfold) that are specialized cells and found in the ileum. M cells will take glucan through pinositosis and took it through the intestinal wall where some cells such as macrophages, T cells, B cells and other immune cells have been waiting. Beta(β)-glucan which phagocytosis by macrophages would be degraded into fragments, and then transported to a bone marrow where fragments-glucan degradation results will be released.

According to Chan *et al.,* (2009), these fragments were arrested by the complement receptor (CR3) which located at the cell surface of granulocytes, monocytes, and dendritic cells. These cells with antibodies then activated. Beta (β)-glucan will bind to macrophage on the CR3 receptor, it is combination receptor that has two binding regions. The first area is responsible for binding the type of complement, a soluble blood protein called C3 (or iC3b). C3 will be attached to the specific antibodies then bind to the targeted pathogen and do opsonisasi. The second area in CR3 binding to carbohydrate receptors on cells of yeast or fungus (PSK) that allows macrophages to recognize yeast as "nonself" [14]. From the second signal of PSK, it can help the process of phagocytosis of macrophages in tuberculosis infection.

The highest of OD value was found for the IgM isotype in all treatment groups. Immunization of *Mycobacterium tuberculosis* in live cell form and are conducted twice within an interval of one week makes the immune responses which occured is still primary immune response. According Bellanti (1993), antibodies can be detected after 10 to 14 days after injection of bacterial cells. The first meeting with the bacteria will raise primary immune response. Immune response which raised by imunogen is dominated by IgM.

OD or absorbance values with the second highest concentration in the (K-), P1, P2 and P3 is IgA. High concentration of IgA in serum according to the statement Baratawidjaja (2006) which states that high IgA levels in serum will be found in respiratory and gastrointestinal infections, like tuberculosis. This is supported by Frank (1995) which states that IgA has functions in early antiviral and antibacterial defense by preventing bacterial adhesion to the mucous membranes.

Subclass IgG2b has a higher concentration may be caused by its ability to bind antigens with a form of protein. According to Scott *et al.* (1990), IgG2a, and IgG2b in mice with IgG1, and IgG3 of human have similarities in their ability to embed complement and protein antigens. Polysaccharide krestin (PSK) is a complex polysaccharide binding protein [21] and *Mycobacterium tuberculosis* is a bacterium which contains several proteins that bind to lipids [15]. So, they make subclass of IgG26 has a higher concentration.

According to Scott *et al.* (1990), IgG3 of mice and IgG2 humans have similarity in recognition of carbohydrate epitopes. The existence of higher enough concentration of IgG3 indicated that PSK take a role in increasing the types

of that immunoglobulin. According to Robinson (1995), Beta (β)-glucan is a natural polysaccharide derivatives which having 7-10 monosaccharide units that are classified into the oligosaccharide. Monosaccharide of PSK consists of glucose (74.6%), mannose (15,5%) xylose (4.8% of), galactose (2.7% of), and high fructose (2.4% of) [28]. IgG2a and IgG1 subclass had the lowest concentration in the serum of treatment group, this probably occurred because IgG1 more capable of binding mast cells[25].

Immunoglobulin light chains are divided into two types, namely kappa light (κ) and lambda (λ) chains. According to Tizard (1987) and Bellanti (1993), the ratio between the kappa and lambda light chains are highly variable among species and their combinations are normally present in each individual. Ig isotype highest with total 6.280 is founded on providing PSK before and after infection with *Mycobacterium tuberculosis*. This indicates the role of providing PSK with different times on Ig isotype of mice which infected by *Mycobacterium tuberculosis*.

Provision of PSK before infection with Mycobacterium tuberculosis has function as prevention (preventive) that encourage to increase the number of lymphocytes formation then increases levels of immunoglobulin more optimally, so that levels of immunoglobulin against Mycobacterium tuberculosis infection will further increases and will be further improved with the provision of PSK after Mycobacterium tuberculosis infection as a treatment (curative). Polysaccharide krestin is expected to prepare and boost immunity against disease that will enter the body. Pietro (2003) states that β-glucan is more effective for prevention (preventive) and treatment (curative) of diseases in related with immune system durability.

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