

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## The Role of Probiotic *Lactobacillus plantarum* IS-10506 toward the Total of CD4+, IgA for the Immune Response of Elderly People.

Sunarto Kadir\*.

Doctor of Health Sciences, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia.

### ABSTRACT

Most of probiotics are used as a therapeutic modality and food supplement for improving the system of body immunity. However, it has not been known that the mechanism of probiotic immunomodulation effect at the system of elderly people's immunity. Moreover, this study aimed at analyzing the effect of probiotic *Lactobacillus plantarum* IS-10506 toward the immune response of CD4+ and IgA in infection model in giving LPS to the mice (*Rattus norvegicus*) strain elderly Wistar instead of healthy elderly mice. Furthermore, this study is an experimental study by using the randomized post test only control group design. The subject of this study was male mice that consisted of 4 groups based on treatment given which were a group that was given placebo (control) in first day until ninth day, a group that was given LPS in first day, a group that was given probiotic in third day until ninth day, and a group that was given LPS in first day and probiotic in third day until ninth day. All of groups were euthanized in tenth day. An examination of immunohistochemistry at the small intestine used specific monoclonal antibody which was mouse anti-Rat CD4+ and IgA. A new finding based on this study was giving probiotic and LPS could increase the content of IgA at the elderly mice, and it could be proven scientifically that IgA as the adaptive immunity in secondary phase, with second stimulation in giving probiotic was occurred switching IgM and IgG became IgA that had high protective power toward pathogen. In conclusion, giving probiotic *Lactobacillus plantarum* IS-10506 and LPS could affect to the rise of immune response that appeared from the expression of immune response such as the rise of CD4+ and IgA in homeostasis condition.

**Keywords:** Probiotic, LPS, CD4+, IgA, adaptive immune, elderly immunity

\*Corresponding author

## INTRODUCTION

At the last 2 decades, there was a rise of elderly population in Indonesia. The proportion of elderly population above 65 years old rose from 1,1% to 6,3% from the total of population. At last 20 years, there was a rise of 5,2 % of elderly population in Indonesia in 1997. This case reflected that the proportion of elderly population would rise twice in 2020 which it would become 28,8 million or 11,34% from all population (Fatmah, 2006).

Significantly, the elder people would undergo mortality and morbidity case more highly than the younger people. The susceptibility of elder people in suffering disease was caused by declining the function of the system of body immunity (Fatmah, 2006). In order to know a change of elderly people's body immunity, it was needed a deep study about immune system which was one of body systems that was affected by the aging process. Moreover, immunocompetence declined in line with the age. It meant that when the people became elder people, the immune system began to lose some functions and it could not respond the stimulus fast or efficiently. A change of immune system which was related to the age was seen to all levels start from chemical change in cell until the difference of protein types which was found on the surface of cell, even until a change of all organs (Whitman, 1999).

One of big changes that were occurred when the body became elder was the process of thymus involution. T cell was lymphocyte population which was very special and very important that had many advantages such as killing the bacteria until helping other cells in immune system. By adding the human's age, thymus suffered atrophy naturally. The elderly people underwent a declining of cell mediated immunity. One of the cells that had a role in cell mediated immunity was CD4+. The cytokine was produced by CD4+ and then, it affected the activity of other immunity cells such as increasing the ability of macrophage in killing pathogen bacteria and stimulating cell B to increase antibody production (Fatmah, 2006).

A decrease of elderly people's immune could make the susceptibility of disease became a basic in searching supplement which was kind of medicine that could help to improve body immunity. One of the supplements was probiotic. Probiotic had been used since long time ago as a treatment for gastrointestinal disorder and as a food supplement. The advantages of probiotic had been researched and been known, but the clear mechanism about how probiotics could improve the immune response of intestinal mucosa was still discussed. Moreover, if probiotic which as living microbiota was given in sufficient quantities, it could give some advantages for the people's health (Fuller, 1989; FAO/WHO, 2001). According to some previous studies based Evidence Based Medicine (*Kedokteran Berbasis Bukti*) showed the efficacy in some clinical conditions.

Probiotic had been proven empirically that it gave many advantages for health. Some advantages of the probiotic that had been proven empirically were such as improving the immune system of mucosa that was very important for the defense of gastrointestinal mucosa (Goldin 1998, Blum and Schiffrin, 2003). Probiotic also had the ability to prevent the growth and adhesion of pathogenic bacteria in gastrointestinal by producing and secreting antimicrobial substances such as bacteriocin and renericyclin. Hence, this probiotic was very important for elderly people. Some organic acid was also produced by probiotic (Fuller et al. 1999; Howard et al. 2001), and it had important role in doing competition in the adhesion place in intestinal mucosa with pathogenic bacteria and it also had functions as a barrier and hastening the elimination of pathogenic bacteria (Suarez et al. 1998; Lim et al. 2000; Sansonetti 2006). Moreover, probiotic had active biological molecule such as peptidoglycan and teichoic acid. This active substance was microbial-associated molecular patterns (MAMPs), that perhaps, this probiotic was known by PRRS (*pattern recognition receptors*) in TLR2 and TLR4 (Sakane, Nezu et al. 2005). Hence, probiotic could have a role as extracellular stimulator by ERK1/JNK MAP kinase that would induce the factor of intracellular transcription which helped the process translational synthesis of certain protein.

Furthermore, probiotic could stimulate the innate immune system even adaptive immune system. The dendritic cell and macrophage could have a role of APC (*Antigen Presenting Cell*). Then, this APC interacted with lymphocyte in the gastrointestinal canal that was known as GALT (*Gut Associated Lymphoid Tissue*). The origin of antigen of microbe cell was known as T cell through MHC-II that was expressed on APC in order to activate CD4+ (Th). CD4+ activated Th1 cell and then, it produced cytokine such as IFN- $\gamma$  and IL-2. This cytokine was the important part in immune response that was intervened by cell. IFN- $\gamma$  stimulated macrophage and IL-2 stimulated T cell of cytotoxic.

This cytokine was part of immune system that was the defense toward invasion of pathogenic bacteria. Hence, the infection could be prevented. This case would affect the condition of infection that occurred more to the elderly people. The novel of isolate probiotic of local-original curd that was researched more was *Lactobacillus plantarum* IS-10506 that had accession number DQ860148. Based on the fact that there was clinical improvement of diarrhea patient who got *Lactobacillus plantarum* IS strain 10506 (LIS 10506) and strain 20506 (LIS 20506) on the improvement of protein constituent of brush border (Ranuh, 2008).

A researched for the human as Valeur et al stated (2004) was observing the distribution of *L. reuteri* in the human's gastrointestinal canal and it was known that *L. reuteri* stimulated the growth of CD4<sup>+</sup> in small intestine. Then, CD4<sup>+</sup> would differentiate to be Th1 or Th2 subset set. After that, it produced IFN- $\gamma$  to activate the phagocyte of macrophage. Whereas, the cell of CD4<sup>+</sup> that differentiated to be Th2 cell would remove IL-4 and IL-5 that could help to differentiate B cell became plasma cell which secreted antibody.

The role of probiotic in several studies on healthy individual toward the efficiency of immune response of mucosa explained more about the efficiency of immune response in the mechanism of IgA adaptive immunity that had promotive and preventive character on intestinal mucosa from the pathogenic exposure (Perdigon et al., 1998; Fang et al., 2000; Isolauri et al., 2005; Dogi et al., 2008). Whether, Galdeano (2007) stated clearly that the mechanism of immune response of mucosa that was modulated by probiotic was through innate immunity (Galdeano et al., 2006; Galdano et al., 2007).

The providing of Lipopolysaccharide (LPS) was intended as infection. Moreover, this study used mice as the experimental animal. The providing LPS was from *Escherichia coli* that would improve the inflammatory response, hence, it stimulated the dismissal of TGF- $\beta$  and IL-10, and finally it would increase the concentration of IgA, IgM, IgE, and IgG in intestinal mucosa. Besides, LPS also stimulated the formation of proinflammatory cytokine such as tumor necrosis factor alpha (TNF $\alpha$ ) and interleukin-6 (IL-6). Hence, it stimulated more to the balance of Th1 cell response by increasing the secretion of IFN- $\gamma$ . Whether, the providing of Cholera toxin stimulated more to the response of Th2 cell on mucosa (Ronco, 2000; Alexander, 2001; Perdigon, 2002).

Therefore, by considering the importance of improving the role or the content of CD4<sup>+</sup>, and IgA on the improvement of elderly people's immune response, the writer of this study was interested to research "The Role of Probiotic *Lactobacillus plantarum* IS-10506 toward the Total of CD4<sup>+</sup>, IgA for the Immune Response of Elderly People".

#### METHOD OF THE STUDY

This study was an experimental study by using the randomized post test only control group design. The population of this study was mice (*Rattus norvegicus* strain Wistar). The sample of this study was twenty mice (*Rattus norvegicus* strain Wistar) that had  $\pm$  11 months of age, between 300-400 grams of weight, and they were suitable with the elderly criteria. Moreover, they were from Surabaya Veterinaria due to the relatively small weight change during the research (Smith, dan Mangkoewidjojo, 1988). The quantity of sample was 5 for each group. In this study, there were four groups. Therefore, the quantity of all samples was 20 mice. Before they were used as the subject of this study, the writer did a clinical evaluation and they were conditioned in appropriate environment with adaptation period in 7 x 24 hours to make sure that the animals did not suffer a disease even did not have potency to spread the disease. Besides, they were given ration as similar as in the treatment of this study. Before having treatment during the study, the writer did screening with some inclusion criteria:  $\pm$  11 month of age, healthy mice that were indicated with their active movement, having shiny eyes, having no dull fur, male, and their weight were between 300-400 grams.

The variable of this study was divided by independent variables which were probiotic *Lactobacillus plantarum* IS-10506 and LPS, dependent variable which was the total of cell that expressed CD4<sup>+</sup> and IgA, and control variables which were the dose of giving probiotic and LPS, the way in giving probiotic and LPS, mice's stress factor, the experimental mice (*Rattus norvegicus* strain Wistar), mice's stable, mice breeding, and the method of examining.

Besides using mice as the experimental animal of this study, the writer also used oral probiotic substance of *Lactobacillus plantarum* IS strain 10506 which had accession number DQ860148: the original probiotic in Indonesia. The probiotic was given in dose of 10<sup>10</sup> cfu/day which was each mouse would get dose

of 1 gram for a mouse/day. This probiotic was diluted in media of NaCl in 2 cc and given through gastric sonde everyday (once in a day) for 7 days for third and fourth group. Moreover, it was given in third day until ninth day. Besides, another substance *Lipopolysaccharide* (LPS) (*Escherichia coli* LPS serotype O55; B5, catalog number: L5418, Sigma Chemical co), Singapore. LPS was given with the dose of 250 µg/kgBB. This LPS was diluted with the emulsion of NaCl 0,9% with the rate of 10:1 and it would be given through gastric sonde in first day only once for second and fourth group. The immunohistochemistry that was used were ether, formalin (with buffer), Hydrogen peroxide, antibody diluent, xylol, ethanol, peroxide, trypsin, streptavidin, distilled water. The analysis of immunohistochemistry used specific monoclonal antibody which were Anti-Rat CD4+ and Anti-Rat IgA. The coloration of immunohistochemistry was used to examine CD4+ and IgA.

This study was done in Laboratory of Biochemistry in faculty of medical science, Universitas Airlangga / RSU Dr. Soetomo, Surabaya. The analysis of immunohistochemistry was done in UPT of Electron Microscopy, Universitas Airlangga Surabaya. The time that was needed to do this study was consisted of adaptation phase in a week in the experimental animal's stable of Laboratory of Biochemistry, Universitas Airlangga Surabaya, the phase of treatment and observation the symptoms in ten days in the experimental animal's stable of Laboratory of Biochemistry, Universitas Airlangga Surabaya, and finally the research phase in the laboratory (toward the animal tissue) which was histochemical observation of small intestine that was done in Biochemical Laboratory of UPT of Electron Microscopy, Universitas Airlangga Surabaya.

After passing the qualification test of research ethics, the writer did the procedure of adaptation phase for the mice for a week with the stable environment in the laboratory and the treatment was done appropriately with the group settled. The first and second groups were the groups which were not given probiotic. First group was a negative control group that was not given LPS and probiotic, however, it was given placebo for nine days. Then, a treatment for second group in positive control that was given LPS (75µg/mouse) was *Escherichia coli* LPS serotype O55;B5 in first day and it was not given probiotic. Whether, the third group was a group that was given probiotic *Lactobacillus plantarum* IS-10506 and it was not given LPS since in third day until ninth day. The fourth group was a group that was given probiotic *Lactobacillus plantarum* IS-10506 and given LPS in first day, then, it was given again the probiotic *Lactobacillus plantarum* IS strain 10506 in third day until ninth day (for 7 days sequentially). The mice were sacrificed after getting anesthetic with ether in tenth day in the morning in order to the removal of small intestine (ileum dan jejunum). When the experimental mice were assured dead, the writer did necropsy by taking the organ that were needed such as small intestine (ileum and jejunum). The small intestine were cleaned and saved temporary with formalin (with buffer) before doing saving and processing the tissues. The organs that were preserved were processed and fixed so that it could be done the advanced treatment. The process in making histological preparation was by the phase of dehydration, clearing, impregnation, and embedding. Moreover, paraffin method was chosen in order to the fixation from this tissue. The result was the writer did the analysis in immunohistochemistry with utilizing specific monoclonal antibody in order to know the rise of the expression total of specific immune system such as CD4+ cell and the expression total of cell in producing IgA on the mucosa cell in small intestine. The result was grouped and analyzed for each group.

The data were obtained as the result of study collected in primary data. Whether, for examining CD4+ and IgA with immunohistochemistry was done in UPT of Electron Microscopy, Universitas Airlangga Surabaya.

Furthermore, the data analysis of this study utilized normality and homogeneity test in order to determine parametric / non-parametric statistical test with Shapiro-Wilk test. The inferential analysis was utilized in order to know the difference that was emerged by given treatment with utilizing Anova. If there was a significant difference, then, it was continued with LSD (*Least Significant Difference*) test in order to know the difference between control groups and each treatment group. The data analysis was analyzed by utilizing the certainty level of 95% ( $\alpha = 0,05$ ).

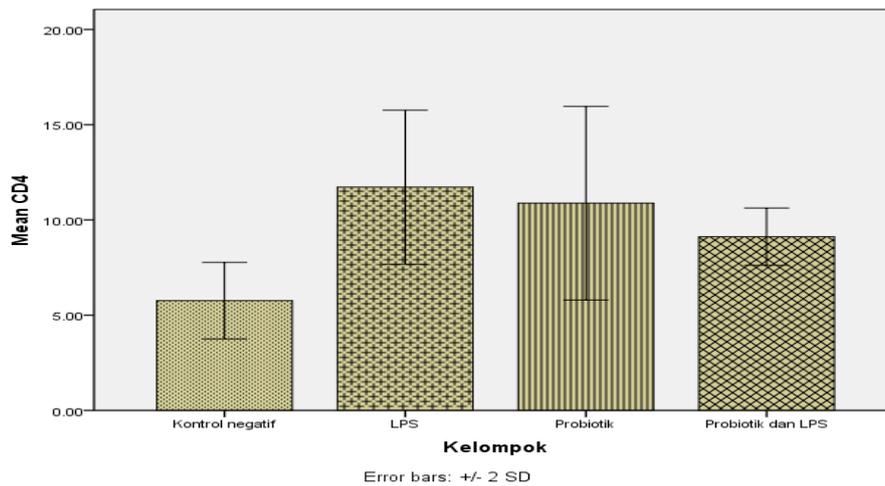
## RESULT AND DISCUSSION

The result of homogeneity test, the data showed that there was no difference between the first and last weight at the experimental mice which were used in this study. Moreover, it meant that the weight factor was not a factor that affected the result of the study.

**Table 1: Description of Specific Immune Response of CD4+ cell**

	N	Mean	Std. Deviation
K-	5	5,76	1,01
LPS	5	11,72	2,02
Probiotic	5	10,88	2,54
Probiotic and LPS	5	9,12	0,75
Rerata Total	20	9,37	2,84

A further description of CD4+ at all groups could be seen as follows:

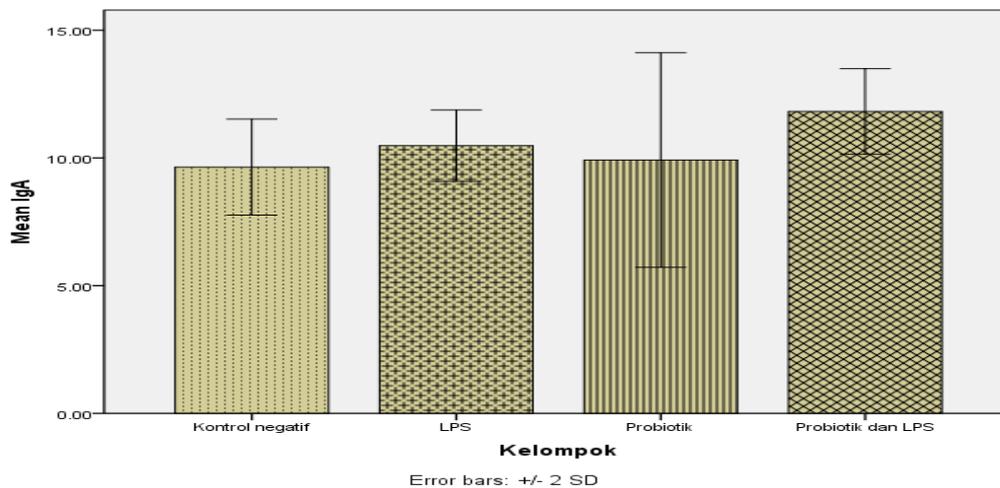


**Picture 1: The expression result of specific immune response of CD4+ cell at all groups**

**Table 2: Description of the Expression Total of Cell in Producing IgA**

	N	Mean	Std. Deviation
K-	5	9,64	0,94
LPS	5	10,48	0,70
Probiotic	5	9,92	2,10
Probiotic and LPS	5	11,82	0,84
Rerata Total	20	10,47	1,45

A further description of the expression total of cell in producing IgA at all groups could be seen as follows:



**Picture 2: The Expression Total of Cell in Producing IgA at all groups**

According to the result, the highest expression total of cell in producing IgA was in a group that was given probiotic and LPS.

**Test of Normality**

The result of calculation by using Saphiro wilk test showed all the data that had normal distribution, except in CD4+ of probiotic group and CD4+ in probiotic group and LPS. Hence, in the next step, the processing of CD4+ could not utilize parametric statistical test with Anova, but it utilized Kruskal Wallis. The result of homogeneity showed a variation of all the data of marker in immune response of homogeneous IgA with value  $p > 0.04$ . These data showed that there was no difference variation of data between both markers.

**Test of Anova**

**Table 3: The Analysis Result of Anova dan Kruskal Wallis (KW)**

	F	KW	Sig,
IgA	3,317		0,068
CD4		12,379	0,006*

The analysis result by using Anova (IgA) in the data that had normal distribution and Kruskal wallis (CD4+) in the data that had abnormal distribution showed that IgA was not significant different ( $p > 0,05$ ). Then, for CD4+ showed the result of significant different ( $p < 0.05$ ).

The test result of Anova and Kruskal Wallis showed the result that had significant different effect of probiotic. In CD4+, the difference happened due to the increasing of CD4+ marker. Meanwhile, in IgA, the difference between the groups was not significant.

In CD4+ showed the significant different, hence, it needed to be processed by using advanced LSD test on parametric statistic and Mann Whitney on non-parametric statistic. The complete test result of LSD and Mann Whitney as follows:

**Table 4: The Analysis Result of Post Hoc**

	Mann Whitney in CD4+
Negative K	5,76 <sup>a</sup>
LPS	11,72 <sup>b</sup>
Probiotic	10,88 <sup>bc</sup>
Probiotic and LPS	9,12 <sup>cd</sup>

Explanation of a,b,c,d = the same notation showed the result that was significant different.

**The Influence of Probiotic toward Immune Response of CD4+**

The result showed marker in CD4+ which was significant different between group of healthy elderly mice, infection model, elderly mice which were given probiotic and elderly mice in infection model which were given probiotic. Among those three treatments, the most influence significant of CD4+ marker was giving LPS and the most responsive immune response. LPS was considered as unidentified thing that was pathogen that entered into the elderly mice's body. Because it was considered as unidentified thing, it would be occurred the process of phagocytosis from response of innate immunity. Then, LPS bacterial endotoxin was strong stimulator for stimulating innate immunity (Alexander, 2001). Westphal dan Luderith (1954) stated that component of lipid A was immunostimulatory or component of main endotoxin from LPS. The stimulation from immune cell by LPS would encourage reaction of intracellular signaling which caused production and secretion from cytokine and other inflammation mediators. LPS was also strong induction of CD4+ in limph node and lien in in-vivo. The increasing of CD4+ in giving LPS showed that this thing would activate macrophage cell, so it stimulated cellular immunity where there was a change from T cell into CD4+. LPS was

component of endotoxin from extracellular bacteria that would be responded by CD4+ T lymphocyte. This process happened when there was attraction from pathogenic bacteria; CD4+ would activate Th 1 cell and Th2 that was continued to produce cytokine. Th1 would produce IFN- $\gamma$ , IL-4 and IL-5 that became an important part in immune response. IFN- $\gamma$  could stimulate macrophage, while IL-4 and IL-5 helped in the differentiation of B cell became plasma cell which gave secretion that became antibody.

Several T cells which were found in thymus and blood circulation were memory T cell and naive T cell. Naive T cell was quiet cell and never touched by strange antigen, meanwhile, memory T cell was active cell which was touched by antigen. When antigen was getting in, naive T cell would be active and it would stimulate immune system for vanishing strange antigen from the inside of body, then it would change to become memory T cell. Afterwards, memory T cell would not be active, then, it would be active again if it faced the same antigen. In group of elderly mice, almost there was no naive T cell because the declining of T cell production by thymus gland along with the age addition. As a consequence, the reserve of naive T cell would decrease and the immune system could not respond responsively to the younger group (Aspinall in Fatmah, 2006).

Significant probiotic increased an expression of immune response in CD4+ marker in LSD result test. The probiotic was bacterial positive gram. The existence of significant increase could impact toward the giving of probiotic. It could be happened because probiotic bacteria were normal flora and all of them had known each other with the host of immune system (Corthesy, 2007). This condition was in line with the research that had done by Galdeano et al. (2006) that probiotic affected the total of CD4+ as histology. This result proved that the giving of probiotic orally would stimulate the intestinal immune mucosa cell to let proinflammatory cytokine (Galdeano, 2006). This increase happened due to good activation from intestinal immune mucosa cell, particularly macrophage and dendrites cell which were involved in innate immunity before, hence, it affected CD4+ cell.

#### **The Influence of Probiotic toward the Expression Total of IgA in Producing Cell**

The result of the study showed that expression of IgA cell was no significant and it tended to increase in giving LPS and probiotic. It could happen because IgA marker was immunity system that was adaptive in secondary phase. Based on the procedure of this study in first day, mice obtained LPS and then in third day, they obtained probiotic. In giving first LPS, the immune system in the body would respond as the body defense in primary phase. In primary phase, the body would respond LPS as the pathogenic bacteria. After having contact with antigen, dendrite cell from lymphoid (and Langerhand cell) would induce the activation of T lymphocyte. The great number of antibody that would be formed in this phase was IgM.

In third day, mice obtained probiotic. Because in first day the body had known LPS as pathogenic bacteria, hence, the giving of probiotic in third day, the mice's body had saved memory, then, it was occurred secondary defense. This was called as secondary response because it related to memory cell after the giving of LPS antigen in first day. Secondary response could be varieties between 10 until 50 times higher than primary response and it worked more long time. In ninth day until tenth day, the increase of IgA could be seen.

In this section, macrophage gave an important role in the process of immunoglobulin antigen which was formed in secondary response that was IgA, because IgA would be more seen in all mucosa such as *tractus respiratorius*, *digestivus* or *urinarius*. While in this research, small intestine played as a part of *tractus digestivus* (Weir, 1990: 49-50).

The process of immune response of IgA was started by the activation of T cell in inducing the release of the total of cytokine and chemokine which had role in the process of B cell activation, isotype switching and expression of specific integrin in antigen-sensitized-8 cells (Cebra, 1999; Mayer, 2003). The process of isotype switching B cell into IgA-producing plasma cell started from mucosa induction. This switching process needed specific signal through the molecule of kostimulator included cytokine and TH cell. This process was influenced by Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) which was produced by Th3 cell. Th2 cell produced IL6. TGF- $\beta$  and IL-10 were cytokines which induced isotype switching from IgM into IgA by B cell (Mayer, 2005; Ezendam, 2005).

The synthesis immunoglobulin process, especially IgA and IgM were started by antigen sampling by M cell and/or dendrites cell (DC) in intestinal mucosa. Antigen would be arrested by receptor in the surface of

dendrites cell. Depending on PAMPs from antigen, dendrites cell would express TLR which was main censor toward different pathogen. Probiotic that considered as micro biota would get in as antigen, it would be arrested by M cell in the mucosa surface and it would be presented by the antigen of presenting cell (macrophage and cell) to either immature B cell or immature T cell (Matsuzaki, 1999; Bland, 2006). Macrophage and cell are inductive sites and they played a role to regulate the humoral and cellular immune response for protection in mucosa.

Activated T cell would differentiate into CD4+ T helper cell and cytokine secretion. Th1 produced IL-2, IL-3 and interferon  $\gamma$  (IFN $\gamma$ ). While Th2 produced IL-6, CD4+ other helper cell called as Th3 (T reg), would give secretion of TGF- $\beta$  that would help to differentiate antigen of specific B cell for isotype switching from IgM into IgA-producing plasma blast. After having maturation in mesenteric lymph node and through the circulation of lymph and blood of T cell and B cell (Immunoglobulin producing plasma cell) would return to mucosal effectors sites. Here, the process of differentiation was ended by using IL-4, IL-5, IL-6, IL-10, TGF- $\beta$ , B cell became immunoglobulin producing plasma cell that would produce IgA in intestinal mucosa (Bauer, 2005; Bland, 2006; Chorthesy, 2007; Galdeano; 2007).

The giving of LPS in this study would become the factor of specific antigen, so mice were able to produce the expression of IgA cell. The existence of IgA showed that probiotic also played actively in adaptive immune response. The adaptive immune response was conducted by modulating the immune response in mucosa, probiotic bacteria were having an approach for communicating (cross-talk) with host of immune cell by helping to know its receptor or knowing the product of probiotic such as metabolite, the component of wall cell and DNA (Corthesy, 2007). Presentation of antigen from intestines caused the form of cell's product of local immunoglobulin cell without the induction from systemic immune. The local immune response in intestinal mucosa was caused by the interaction between probiotic bacteria, epithelia and immune cell which were combined with lamina propria. Moreover, there were three interactions of intestinal cell in arising immune response, included: through M cell in peyer's patch, presentation and the process of entering antigen until epithelia cell and the interaction of epithelia cell and also the elimination of antigen through portal circulation or induction of local immune response by using cytokine activation. Besides, an important factor of local defense system involved the cell migration from specific B cell and T cell from peyer's patch, (Bouvet, 1999; Collins, 1999; Bland, 2006).

After the process of transitsis, IgA dan IgM would tie with J chain and secretory component; then it became IgA and sIgM. The process of regulation immunoglobulin, especially IgA would involve several cytokine. According to Perdigon (2002), the production of J chain by B cell would also involve cytokine such as IL-2, IL-5 and IL-6. For avoiding more process of inflammation in the activity of cytokine, there was a mechanism of down regulated which was played by TGF- $\beta$ , IL-4 and IL-10 (Perdigon, 2002; Goldeano, 2007).

## CONCLUSION

The giving of probiotic *Lactobacillus plantarum* IS-10506 in mice (*Rattus norvegicus* strain Wistar) in healthy elderly group had significant difference toward CD4+ ( $p < 0,05$ ), it was no significant difference toward IgA ( $p > 0,05$ ), that was still in homeostatic condition. The giving of probiotic *Lactobacillus plantarum* IS-10506 in mice (*Rattus norvegicus* strain Wistar) in healthy elderly group obtained lipopolysaccharide that gave benefit to the immune response significantly and increased the expression of immune response in IgA marker. In this study, it was not conducted the controlling to an adult mice. Then, for the next study, the writer hopes for the other researchers will increase the accurate result because it is needed to differentiate the adult mice and the elderly one. Hence, it can be known further the difference of the influence of probiotic to immune response in elderly group.

## REFERENCES

- [1] Alexander C, Rietschel E, 2001. Bacterial lipopolysaccharides and innate immunity. *J. Endotoxin*; 7: 167-200.
- [2] Anderson W, 1999. Immunology. Edisi 1. Hayes Barton Press; *Edinburgh*:53-65
- [3] Anonymous, 2010. Theories Of Aging.<http://www.antiaging-systems.com /agetheory.htm>
- [4] Arisman, 2007. Gizi dalam Daur Kehidupan. EGC. Jakarta.

- [5] Bauer E, Williams B, Smidt H, et al, 2005. Influence of the Gastrointestinal Microbiota on Development of the Immune System in Young Animals. *Issues Intestinal Micro*; 7: 35-52.
- [6] Bland D, Barrera C, Reyes V, 2006. Gastrointestinal Mucosal Immunology. Dalam: Tying S Eds. *Mucosal Immunology and Virology*. Limmit ed. Springer Verlag London: 23-54.
- [7] Blum S, Schiffrin E, 2003. Intestinal Microflora and Homeostasis of the Mucosal Immune Response: Implications for Probiotic Bacteria?. *Curr Issues Intest.Microbiol* 4: 53-60.
- [8] Cebra JJ, 1999. Influences of microbiota on intestinal immune system development. *Am J Clin Nutr*; 69(5):1046-51.
- [9] Collins M, Gibson G, 1999. Probiotics, prebiotics, and symbiotics : approaches for modulating the microbial ecology of the gut. *Am J Clin Nutr*; 69: 105257.
- [10] Collado, M. C., M. Gueimonde, M. Hernandez, Y. Sanz, and S. Salminen. 2005. Adhesion of selected *Bifidobacterium* strains to human intestinal mucus and its role in enteropathogen exclusion. *J. Food Prot.* 68:2672-2678.
- [11] Collado MC, Gueimonde M, Sanz Y, Salminen S. 2006. Adhesion properties and competitive pathogen exclusion ability of bifidobacteria with acquired acid resistance. *J Food Prot* 69(7):1675-9.
- [12] Collado, M. C., I. S. Surono, J. Meriluoto, and S. Salminen. 2007. Cellsurface properties of indigenous dadih lactic acid bacteria and their interactions with
- [13] pathogens. Submitted for publication.
- [14] Collado MC, Meriluoto J, Salminen S. 2007. Interactions between pathogens and lactic acid bacteria: aggregation properties and adhesion to hydrocarbons. *Eur J Food Res Technol.* in press.
- [15] Corthesy B, Gaskins R, Mercenier A, 2007. Cross-Talk between Probiotic Bacteria and the Host Immune System. *J. Nutr*; 137: 781-90.
- [16] Dachlan Y.P, 2008, Handout Infeksi dan Imunologi, Program Doktor Program Studi Ilmu Kedokteran Pasca Sarjana Unair.
- [17] Dogi C, Galdeano M, Perdigon G, 2008. Gut immune stimulation by non pathogenic Gram(+) and Gram(-) bacteria. Comparison with a probiotic strain. *Cytokine*; 41:223-31.
- [18] Eckmann L, 2006. Animal models of inflammatory bowel disease, lesson from enteric infections. *Ann NY Acad Sci*; 1072: 28-38.
- [19] Endaryanto A, 2007. Imunoregulasi Th1, Treg dan Th2 melalui TLR2 dan TLR4 oleh probiotik LGG dan L plantaru IS-10506 dalam penurunan reaksi alergi. Disertasi.
- [20] Ezendam J, Opperhuizen A, Loveren H, 2005. Immunomodulation by probiotics: efficacy and evaluation. *Current Pharmaceutical Design*: 11 55-74
- [21] Fang HE, Elina T, Heikki A, et al, 2000. Modulation of Humoral Immune Response Through Probiotic Intake. *Immunology and Med Micro*; 29: 4752.
- [22] Fatmah, 2005. Persamaan (Equation) Tinggi Badan Manusia Usia Lanjut (Manula) Berdasarkan Usia dan Etnis pada 6 Panti Terpilih di DKI Jakarta dan Tangerang Tahun 2005. *Makara Kesehatan.* 10 (1): 7-16
- [23] Fatmah, 2006. Respons imunitas yang rendah pada tubuh manusia usia lanjut. *Makara Kesehatan.* 10 (1): 47-53.
- [24] Floch M, Montrose D, 2005. Use of Probiotics : an Analysis of the Literature. *Gastroenterol Clin N Am*; 34: 547-70
- [25] Fujihashi K, McGhee JR, 2005. Th1/Th2/Th3 cells for regulation of mucosal immunity, tolerance, and inflammation. Dalam: Mestecky J, Lam ME, Strober W, Bienenstock J, McGhee JR, Mayer L. *Mucosal Immunology*. Edisi 3. *Elsevier Academic*; London: 539-55.
- [26] Fujii K, Sato R, Goto S, et al, 2007. Prevention of Pathogenic *Escherichia coli* Infection in Mice and Stimulation of Macrophage Activation in Rats by an Oral Administration of Probiotic *Lactobacillus casei* I-5. *Biosci. Biotechnol. Biochem*; 71: 866-73.
- [27] Galdeano M, Le Blanc, Vinderola, et al, 2007. Proposed Model: Mechanisms of Immunomodulation Induced by Probiotic Bacteria. *Clin Vaccine Immunol*; 14: 485-92.
- [28] Galdeano MC, Perdigon G, 2006. The Probiotic Bacterium *Lactobacillus Casei* Induces Activation of the Gut Mucosal Immune System through Innate Immunity. *Clin and Diag Lab Immunology*; 13 (2): 219-26.
- [29] Hambuako, 2008. Jumlah Penduduk Lanjut Usia Meningkat.
- [30] Hanafiah, K.I. (2004). Rancangan Percobaan. Teori dan Aplikasi. K.I. Hanafiah. Jakarta, PT. *Radja Grafindo Persada*: 9-10.
- [31] Hardita I.W., 2004. Sehat Bugar di Usia Lanjut.

- [32] Hartiningsih S, 2008. Peran Status Gizi dan Status Infeksi Pra Suplementasi terhadap Hambatan Peningkatan Kadar Retinol dan Imunitas Pasca Suplementasi ulang Vitamin A Dosis Tinggi pada Balita. Disertasi.
- [33] Isolauri E, Salminen S, 2005. Probiotics, Gut Inflammation and Barrier Function Gastroenterol. *Clin N Am*; 34: 437-50.
- [34] Isolauri E, Siitas Y, Kankaanpaa P, et al, 2001. Probiotics: Effects on Immunity. *Am J Clin Nutr* ; 73: 444-50.
- [35] Isolauri E., et al. 2001. Probiotics: effects on immunity. *Am. J. Clinical Nutrition*. 73(6): 1142S-1146S.
- [36] Isolauri, Kirjavainen P, Salminen S, 2002. Probiotics: a Role in the Treatment of Intestinal Infection and Inflammation. *Gut*; 50 : 54-59.
- [37] Kuntoro H, 2008. Metode Sampling dan Penentuan Besar Sampel. Edisi revisi. Pustaka Melati. Surabaya.
- [38] Laksmiarti, 2002. Tetap Sehat di Usia Lanjut Dengan Gizi Sehat. Puslitbang Pelayanan dan Teknologi Kesehatan Surabaya.
- [39] Lamm M, Quagliata J, 2002. Origin and Homing of Intestinal IgA AntibodySecreting Cells. *J Exp Med*; 195 (2) : 5-8
- [40] Maryam R. S., dkk, 2008. Mengenal Usia Lanjut dan Perawatannya. Jakarta: Salemba Medika.
- [41] Matsumoto T, Kitano A, Oshitani N, et al, 1988 Immunoglobulin-Containing Cells in the Colonic Mucosa of Rabbit with Carrageenan-Induced Colitis. *Dis Colon Rectum*; 31: 723-29.
- [42] Matsuzaki T, Chin J, 1999. Modulating Immune Responses with Probiotic Bacteria. *Immunology and Cell Biology*; 78: 67-73.
- [43] Mayer L, 2003. Mucosal Immunity. *J Ped*; 111: 1595-1600.
- [44] Perdigon G, Alvarez S, Rachid M, et al, 1995. Probiotics Bacteria for Humans: Clinical System for Evaluation of Effectiveness. *J Dairy Sci*; 78: 15971606.
- [45] Perdigon G, Fuller R, Raya R, 2001. Lactid Acid Bacteria and their Effect on the Immune System. *Curr. Issues Intest.Microbiol*; 2(1): 27-42.
- [46] Perdigon G, Galdeno M, Valdez JC, et al, 2002. Interaction of lactic acid bacteria with the gut immune system. *Eur. J.Clin.Nutr*; 56: S21-S26.
- [47] Perdigon G, Vintini E, Alvarez S, et al, 1999. Study of the Possible Mechanism Involved in the Mucosal Immune System Activation by Lactic Acid Bacteria. *J Dairy Sci*; 82: 1108-14.
- [48] Ranuh Gunadi Reza IGM (2008) Ekspresi Protein Galectin-4, Myosin-1a, occluding dan ZO-1 sebagai efek pemberian probiotik *Lactobacillus plantarum* IS pada Perbaikan Kerusakan Brush Border Usus Akibat Lipolysaccharide *E. coli*. Disertasi.
- [49] Ronco J, Guy B, 2000. Adjuvants for mucosal vaccines. Dalam: Fuller R, Perdigon G, Eds. Probiotics 3, Immunomodulation by the gut microflora and probiotics. *Kluwer Academic Publishers London*: 29-37.
- [50] Smith B. John B. V. Sc, Mangkoedjojo Soesanto, 1988. Pemeliharaan, Pembiakan dan Penggunaan Hewan Percobaan di Daerah Tropis. Penerbit Universitas Indonesia. UI Press.
- [51] Sudiana IK, 2005. Teknik pemeriksaan imunohistokimia. Dalam Sudiana IK, ed. Teknologi ilmu jaringan dan imunohistokimia. Cetakan pertama.Jakarta Sagung Seto; h: 36-46
- [52] Surono, I.S. (2003). "In vitro probiotic properties of indigenous dadih lactic acid bacteria". *Asian-Aus. J. Anim. Sci* 16:726-731
- [53] Surono, I.S. and A. Hosono (1996). "Antimutagenicity of milk cultured with lactic acid bacteria from dadih against mutagenic ferassi". *Milchwissenschaft* 51:493-497.
- [54] Vinderola G, Matar C, Perdigon G, et al, 2005. Role of Intestinal Epithel Cells in Immune Effects Mediated by Gram-Positive Probiotic Bacteria:Involvement of Toll-Like Receptors. *Clin and Diagn Lab Immunol*; 12: 1075-84.
- [55] Vinderola G, Perdigon G, Duarte J, et al, 2006. Effects of the oral administration of the exopolysaccharide produced by *Lactobacillus kefiranofaciens* on the gut mucosal immunity. *Cytokine*; 36; 254-60.
- [56] Weid T, Bulliard C, Schiffrin EJ, 2001. Induction by a lactic acid bacterium of a population of CD4<sup>+</sup> T cell with low proliferative capacity that produce transforming growth factor-R and interleukin-10. *Clin Diag Lab Immunol*; 8: 695-701.