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Submission date: 31-May-2021 05:20PM (UTC+0700)
Submission ID: 1597742430
File name: 33-61-1-SM.pdf (319.38K)
Word count: 3179
Character count: 18455

RESEARCH ARTICLE

URL of this article: http://heanoti.com/index.php/hn/article/view/hn1216

The Effect of Probiotics *Lactobacillus Reuteri* on Levels of Ifn-Γ (Th1) and Il-10 (Th2) Cytokines Cells in Mice Postpartum Induced by *Staphylococcus Aureus*

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ABSTRACT

The direct causes of maternal mortality among others bleeding, preeclampsia, long delivery, abortion complications, and infections. Nosocomial infections are caused by *Methicillin-Resistant Staphylococcus aureus* (MRSA), obtained from infection *Staphylococcus aureus* and now resistant of antibiotics. The spread of bacterial resistance is existing drugs, pushing for extracting antibacterial source of other materials, such as the use of probiotics. Probiotics is as non-pathogenic microorganisms in food supplementation component, which has health benefits for the prevention and treatment of disease, when it is in sufficient quantities. Probiotics is as immunomodulatory and immunostimulatory, then activate all CD4+ T cells into Th1 (IFN-γ) and Th2 (IL-10) cells.Prove whether the effect of probiotic *Lactobacillus reuteri* to levels of IFN-γ and IL-10 cytokinecells on the spleen of mice model postpartum induced *Stapgylococcus aureus* bacteria.The study uses pure posttest-only randomized control-group experimental design which utilizes 40 mice (*Balb/c*) and gestasional aged 13 days which are divided into 8 sample groups. Dosis *Lactobacillus reuteri* $1x10^{10}$ CFU/mice in 250µl via a oral sonde (a gavaging needle). Dosis *Staphyloccus aureus* $5x10^{7}$ CFU/mice in 200µl via spuit without needle per *vaginal*. The levels of IFN-γ and IL-10 cytokine were analyzed for enzyme linked immunosorbent assay (ELISA) kits. This study showed statistically that is significant in levels of IL-10 cells on the first day postpartum, levels of IL-10 and IFN-γ cytokineon the third day postpartum showed statistically that is no significant. There is influence of probiotic *Lactobacillus reuteri* on levels of IFN-Ɣ and IL-10 cytokine in mice postpartum induced *Staphylococcus aureus*.

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Keyword: *Lactobacillus reuteri*, *Staphylococcus aureus*, IL-10, IFN-γ

INTRODUCTION

Background

The puerperium is the greatest cause of death in mother at 48.17% [1]. The direct causes of maternal mortality among others bleeding (28%), preeclampsia (24%), long delivery, abortion complications, and infections (11%) [2]. Nosocomial infections is caused by *Methicillin-Resistant Staphylococcus aureus* (MRSA) at 40-60%, obtained from infection *Staphylococcus aureus* and now resistant in hospital [3].

Puerperal infection is caused by bacteria in the genital tract after childbirth [6], due to injury to the cervix, vulva, vagina, and perineum during delivery [7]. Some research suggest that bacteria that commonly cause genital tract infection of woman is *Staphylococcus aureus* [6], there is trauma or injury then as a medium for the entry of *Staphylococcus aureus* infection [8] and be colonies [10]. The incubation period in *Staphylococcus aureus* 1-8 hours, in case of toxic shock syndrome occurs in 5 days [9].

Infection *Staphylococcus aureus* be a problem that serious that time, because of the increase in resistance bacteria on various types of antibiotics, such as β-lactam antibiotics, penicillins, cephalosporins, and carbapenem [4], tetracycline, rifampicin, clindamycin and gentamicin (12.6% -> 39%), erythromycin (65.4%) and fluoroquinolones (72.3-85.8%) [5]. The spread of bacterial resistance to existing drugs, pushing for extracting antibacterial source of other materials, such as the use of probiotics. Probiotics is as non-pathogenic microorganisms or bacteria in food supplementation component, it is including the class of bacterial flora, which has health benefits for the prevention and treatment of disease [11], when it is in sufficient quantities [12], and there is in gastrointestinal tract, vagina, breast milk (ASI) [13]. Probiotics is as immunomodulatory on the immune system and immunonutriant to infection [14,15].

Probiotics are consumed by orally, it through barriers that exist in the gastrointestinal tract consisting of mucus. The antigen binds APC to be presented by MHC II was in Plaque Peyer (ileum), then activate all CD4+ T cells into Th1 (IFN-γ) dan Th2 (IL-10). They have been activated will lead to blood circulation and experience the process of homing to mucosal tissues throughout the body, such as mucosal tissue gastrointestinal tract, genitourinary tract, upper and lower respiratory tract, and the gland duct mamae dan lymphoid tissues (spleen and lymph nodes) [16,17].

Cytokines IFN-Ɣ is a very strong macrophage activator that promotes the production of antibody isotype that support phagocytosis of microbes that can inhibit infection. Cytokines IL-10 is a cytokine most produced by activated macrophages, monocytes, cytokines IL-10 has the function of inhibiting Th1 cytokine produced by macrophages [18].

Purpose

The purpose of this research was to know Effect of probiotic *Lactobacillus reuteri* on levels of IFN-Ɣ and IL-10 cytokine in mice postpartum induced *Staphylococcus aureus*.

METHODS

Mice

A total of 40 female BALB/c mice weighing 30 – 35 g and gestational aged 10 days, were obtained from Biology Technology (Biotek) Laboratory, Islamic State University of Malang. Mice were maintained in an automatic light/dark cycle and provided water and chow ad libitum. Mice were acclimatized to the animal facility for 3 days before experimentation. Healthy and Age-matched (13 day old) mice were used in all experiments, which are divided into 8 sample groups. The control group (K1 and K2) received placebo NaCl 0,9%, SA group (K3 and K4) received *Staphyloccus aureus* bacteria, LR group (K5 and K6) received probiotic *Lactobacillus reuteri*, SA-LR group (K7 and K8) received *Staphyloccus aureus* bacteria and probiotic *Lactobacillus reuteri*. These experiments were were performed in accordance with guidelines of the Parasitology Laboratory, University of Brawijaya for animal care.

Bacterial Preparation

Lactobacillus reuteri were purchased originally from the American Type Culture Collection (ATCC#6475) (Manassas, VA 20108 USA). Preparations containing lactobacilli for use in *ex vivo* experiments were prepared from overnight, at 25^0 C (Ph 6.5) in Lactobacilli MRS Broth (Man-Rogosa-Sharpe liquid medium) medium, in which glucose was replaced with sucrose, autoclave at 121^0 C for 15 minutes.

The strain of *Staphylococcus aureus* used in these experiments is a clinical isolate obtained from a blood and pus patient at Saiful Anwar Hospital. *Staphylococcus aureus* has been positive catalase and positive coagulation. Preparations contain*Staphylococcus aureus* in Nutriant Broth medium.

Bacterial Treatment

Naïve mice received 1 x 10^{10}*Lactobacillus reuteri* in 250µl of MRS Borth (Karimi, *et al*., 2009 & Qiao, *et al*., 2015) via a stomach sonde (a gavaging needle) from gestational aged 13 weeks until first postpartum and third postpartum. The inoculating dose of *Staphylococcus aureus* was determined to be 5 x 10^7 in 200µl (Lazarenko, *et al*., 2012) via spuit without needle per *vaginal* in postpartum. Control animals received placebo NaCl 0,9% via spuit without needle per *vaginal* in postpartum. After treatments, animals were killed and spleens removed and processed for fluorescent-activated cell sorter (FACS)/ Flow cytometry analysis or cell isolation.

Cytokines quantification in serum

Mouse IL-10 (Biolegend, USA), and IFN-γ (Biolegend, USA) enzyme linked immunosorbent assay (ELISA) kits were used according to the manufacturers' instructions.

Ethical Clearance

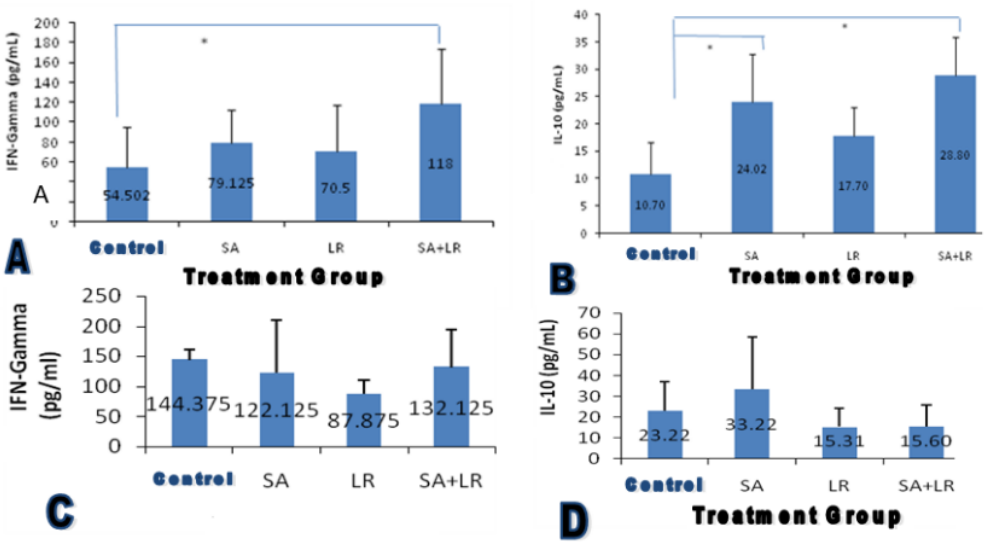
All materials and methods for experiments included in this study were approved by Ethical Committee of Health Research of Health Polytechnic of Ministry of Health Malang, East Java, Indonesia (277/KEPK-POLKESMA/2016).

Statistical analysis

Experimental results are expressed as means ± the standard errors of the means. All data were analyzed using the parametric One Way Anova test, with SPSS LSD software (levels of IL-10 and IFNγ cytokines). A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Result of mean levels of IFN-Ɣ and IL-10 cytokines and shows statistically (Fig. 1A and 1B) probiotic *Lactobacillus reuteri* has effect on levels of IFN-Ɣ and IL-10 cytokines in mice postpartum induced *Staphylococcus aureus* that on the first day postpartum.Result of mean levels of IFN-Ɣ and IL-10 cytokines and shows statistically (Fig. 1C and 1D) probiotic *Lactobacillus reuteri* has no effect on levels of IFN-Ɣ and IL-10 cytokines in mice postpartum induced *Staphylococcus aureus* that on the third day postpartum.



(A) and (B) the first day postpartum; (C) and (D) the third day postpartum,
*($p< 0.05$), **($p< 0.001$), explanation:
Control : The group was given treatment placebo NaCl 0,9%
SA : The group was given treatment *Staphylococcus aureus*
LR : The group was given treatment *Lactobacillus reuteri*
SA+LR : The group was given treatment *Lactobacillus reuteri* + *Staphylococcus aureus*

Figure 1. Levels of IFN-γ and IL-10 cytokines difference between Group Treatment

DISCUSSION

Based on these results the levels of IFN-γ cytokine in H1 on *Lactobacillus reuteri* and *Staphylococcus aureus* (SA + LR) group are significant differences with the control group (Control) (Fig. 1A). This is in line with research that states that gram-positive bacteria Th1 [23], induces the production of cytokines, particularly IL-12 and IFN-γ [24]. Immunological profile in the initial exposure of pathogenic bacteria (*Staphylococcus aureus*) will change the balance of Th1 cell immunological / Th2, which would be an increase Th1 responses, whereas Th2 responses will decline [25]. Exposure to pathogenic bacteria will cause an increase in pro-inflammatory immune response that Th1 and Th17 immune response as well as an emphasis on Th2 immune responses [26,27]. This is supported by the results of other researchers that levels of cytokines IL-17 and Th17 cell percentage in the group of *Staphylococcus aureus* (SA) increased significantly on the first day.

Giving the probiotic *Lactobacillus reuteri* up with postpartum day three and induced *Staphylococcus aureus* causes a decrease in the levels of IFN-γ , as seen in Figure 1C which showed the average levels of IFN-γ

in the group of *Lactobacillus reuteri* and *Staphylococcus aureus* (SA + LR) decreased compared with the control group and *Staphylococcus aureus* (SA). This is consistent with studies in normal mice BALB / c by probiotics for 20 days will lead to an emphasis on the production of Th1 cytokines (IL-2 and IFN-γ) in the spleen and mesenteric lymphonodi cells compared to the control normal [27].

On exposure to *Staphylococcus aureus*, the maximum production of the Th1 immune response (IL-2 and IFN-γ) is 3-6 hours after exposure and then will decline followed a shift towards Th2 immune response (IL-4 and IL-5) [28]. Effect of *Staphylococcus aureus* on the response immune say also depends on the dose given, where the administration of low doses is ± 100µg / ml an immune response of pro-inflammatory (Th1 and Th17) reaches the maximum value at 12 hours after exposure, whereas at high doses ± 1000µg / ml will reach a maximum at 24 hours after exposure, then the immune response decreases [29].

Group of *Staphylococcus aureus* (SA) and were given the probiotic *Lactobacillus reuteri* and induced *Staphylococcus aureus* in H1 increased levels of IL-10 significantly compared with the control group values ($p < 0.05$). This is shown in Figure 1B shows that the average levels of IL-10 group of *Staphylococcus aureus* (SA) and *Lactobacillus reuteri* and *Staphylococcus aureus* (SA + LR) is higher than the other groups. This is in line with research that states that the LPS can cause cell producing cytokines IL-5 and IL-10 is very dominant compared to cytokine-producing cells others [30]. IL-10 is considered as an anti-inflammatory cytokine produced by T cells upon infection of *Staphylococcus aureus* which serves to lower the performance of IFN-γ by decreasing macrophage activation [30]. This is consistent with the results in this study that the group of *Staphylococcus aureus* (SA) levels of IL-10 increased significantly compared with the control group.

Results other studies state that *Lactobacillus reuteri* ATCC 6475 proved to suppress pro-inflammatory cytokine IL17 mediated by increased production of anti-inflammatory cytokine IL-10 through the activation of histamine from receptor H2 [31]. This is consistent with research in which the *Lactobacillus reuteri* has anti-inflammatory effect that will reduce production proinflammatory cytokines by splenocytes and cells of Peyer's patches, but do not increase the production of IL-10 [32].

Provision of *Lactobacillus reuteri* in mice induced parturition *Staphylococcus aureus* in this study lead to decreased levels of IL-10 on the third day post-partum (Fig. 1D). This is in line with research that states that the provision L.rhamnosus in patients with Crohn's disease will decrease the production of IL 10 [33]. It is also obtained from the results of other studies that stated that a decline in the number of secretions cytokine IL-5 as a Th2 cell immune response in LPS group –probiotic [11].

CONCLUSION

There is influence of probiotic *Lactobacillus reuteri* on levels of IFN-Ɣ and IL-10 cytokine in mice postpartum induced *Staphylococcus aureus*.

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