KARYA TULIS ILMIAH

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Oleh:

Tri Yudani M. Raras
Desie Dwi Wisudanti
Hetti Rusmini

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GOAT KEFIR STIMULATE THE SHIFTING OF TH2 CELLS IN HEALTHY PERSON

Tri Yudani M. Raras[1], Desie Dwi Wisudanti[2], Hetti Rusmini[3]

[1]*Department of Biochemistry and Molecular Biology, Faculty of Medicine, Brawijaya University, Malang*
[2]*Department of Pharmacology, Faculty of Medicine, University of Jember, Jember*
[3]*Department of Farmacology, Faculty of Medicine, Malahayati University, Lampung*
Corresponding author: rarastriyudani@gmail.com

Abstract

Kefir, one of fermented milk product consist of a mixture of yeast and bacteria has been known to promote health. Several studies on mice demonstrated that kefir may play a role as immunomodulator. However only few of them explore how kefir stimulate immune cells as well as cytokines that involve in innate immune system directly in human. This study aims to observe the immunomodulatory effect of kefir in vitro in healthy individuals. We measured the lymphocytes T $CD4^+$ and $CD8^+$ cells and secretion of Th1 and Th2 cells cytokine. We used kefir from goat milk with maximal age of 1 month after fermentation process. Eleven Volunteers were recruited after being informed about the study and signing informed consent. Peripheral Blood Mononuclear Cells (PBMCs) were isolated from 12 ml blood of normal healthy individuals. As many as 5.10^5 cells were cultured after subjected with addition different concentration of kefir: 5% (P1), 2% (P2), 1% (P3) and 0.5% (P4). PPD served as positi ve control (K+). Percentages of $CD4^+$ and $CD8^+$ T lymphocytes were measured by flowcytometer. Expression of IL-2 and IL-10 levels were determined by ELISA method. The measurement of $CD4^+$ and $CD8^+$ T cell demonstrated that there was no difference in both groups ($p = 0.178$ and $p = 0.438$) compared to K- as well as K+. In contrast the IL-2 levels showed significantly different between K- with K+ ($p = 0.002$), K+ with P2 ($p = 0.002$), P3 ($p = 0.001$) and P4 ($p = 0.007$). Finally the concentration of IL-10 were significantly different between K- with K+ ($p = 0.008$), K- with P1 ($p = 0.004$), P2 ($p = 0.006$), P3 ($p = 0.004$) and P4 ($p = 0.017$). Low concentration of kefir apparently modulate immune system of healthy person by shifting the immune response towards Th-2 cells through increasing level of IL-10.

Keywords: $CD4^+$, $CD8^+$, Kefir, IL-2, IL-10, immunomodulator

INTRODUCTION

Kefir is a fermented milk probiotic that has been widely known to promote health. This is not only due to its nutrition value[1], but also because of their function as an anti hypercholesterolemia,[1,2] antimicrobial,[3] as well as its immunomodulatory effect.[4] Kefir was also act as a probiotic on gut microbes or boost immune system function.[5]

Immunomodulators agent is defined as a substance that has potency to enhance immune system by stimulation (immunostimulant) or repress the abnormal immune response (immunosuppressants). Those two dual character can be assessed by the ability of agent to stimulate increased levels of lymphocytes T CD4 + and CD8 +, secretion of Th1 and Th2 cells cytokines. This study aims to observe the immunomodulatory effect of kefir *in vitro* in healthy individuals. We measured the lymphocytes T CD4+ and CD8+ and secretion of cytokines IL-2 (Th1) and IL-10 (Th2) cells.

MATERIALS AND METHODS

Sample Preparation Peripheral Blood Mononuclear Cells (PBMCs) were isolated from 12 ml blood of normal healthy individuals. Subject divided into 5 groups concentration of kefir: 5% (P1), 2% (P2),

1% (P3) and 0.5% (P4). without kefir (K-) PPD served as positive control (K+). This study was approved by the Ethics Committee of Faculty Medicine of Brawijaya University Malang and informed consent has been obtained from all individuals.

Culture for Lymphocytes and Kefir Treatment Lymphocytes were isolated from peripheral venous blood by adding Lymphocyte Separation Medium (Sigma-Aldrich.Co, USA) following density centrifugation at 1200xg for 30 min. The ring that was formed was then isolated and counted. As many as 5.10^5 peripheral blood cells were suspended in 300 µl medium RPMI 1640 (Sigma-Aldrich, USA) containing 2 mM glutamin (Gybco, USA), Penicilin-streptomicin 10.000 U/ml (Gybco, USA), and 10% Fetal Bovine Serum(Gybco, USA), then incubated for four days at 37°C with 5% CO^2. Cells were cultured after supplementation of different concentration of kefir: 5% (P1), 2% (P2), 1% (P3) and 0.5% (P4).

Flowcytometry Analysis At day 4, cells were harvested. Cells were labeled with FITC anti human CD4 (Biolegend, USA), FITC anti human CD8 (Biolegend, USA). The percentage of CD4 and CD8 was measured using *flowcytometer* (BD, USA).

Enzyme-Linked Immunoabsorbent Assay (ELISA) Supernatans from CD4 T cells were collected for IL-2 and IL-10 cytokines measurements using IL-2 and IL-10 ELISA kit (Biolegend, USA).

Statistic Analysis Differences response of kefir treatment were determined by *one-way* ANOVA *(Analyse of Variance)* ($p < 0,05$). Statistical analysis was performed using SPSS 20.

RESULTS

The measurement of $CD4^+$ and $CD8^+$ T cell demonstrated that kefir could not stimulate the production of both T cell type. Because the concentration of T cell after supplementation with kefir was not different co mpared to negative control. Although the concentration of $CD8^+$ T cell looks higher than $CD4^+$ T cell however there was no statistical different between both groups ($p = 0.178$ and $p = 0.438$) (figure 1).

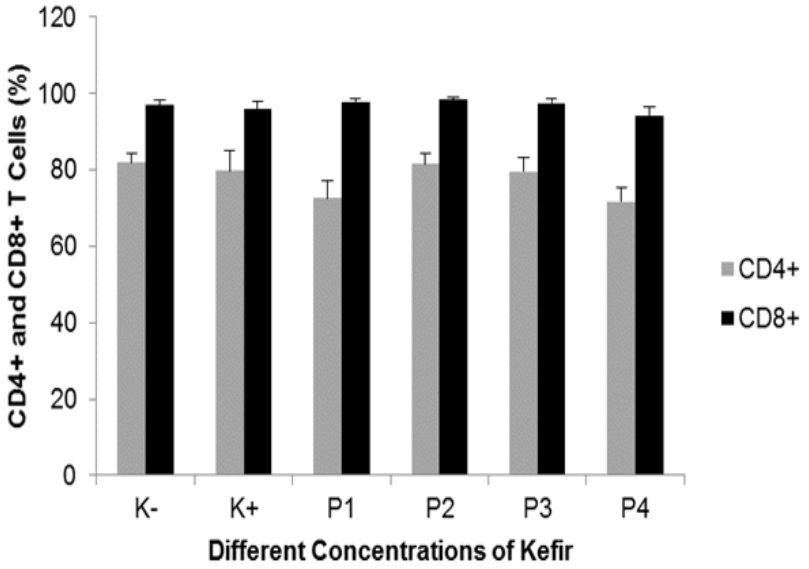

FIGURE 1. Measurement of CD4 and CD8 T cell from PBMC healthy person after supplementation with different concentration of kefir : 0% (K-), 5% (P1), 2% (P2), 1% (P3) and 0.5% (P4). PPD served as K+.

The expression of Th2 cell cytokine IL-10 levels exceeded beyond the production of Th1 cell cytokine IL-2 in all treated groups ($p= 0.002$). The level of cytokine IL-10 was significantly different between

K- with K+ (p = 0.008), K- with P1 (p = 0.004), P2 (p = 0.006), P3 (p = 0.004) and P4 (p = 0.017). (figure 2). The Th1 cell cytokine IL-2 itself remained unchanged after stimulation with kefir. The concentration in all groups were as low as negative control (K-) that means without stimulation of kefir (figure 2).

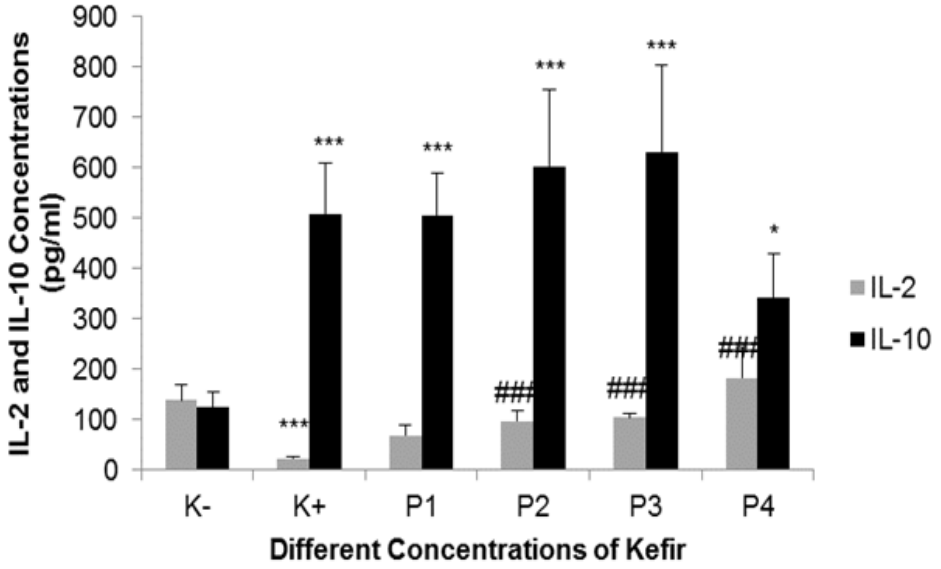

FIGURE 2. Measurement of cytokine IL-2 and IL-10 from PBMC healthy person after supplementation with different concentration of kefir : 0% (K-), 5% (P1), 2% (P2), 1% (P3) and 0.5% (P4). PPD served as K+.

DISCUSSION

Application of kefir on peripheral blood of healthy individual apparently did not significantly stimulate the production of CD4+ T and CD8+ T cells. However, it did increase the secretion of Th-2 cell cytokine IL-10 rather than Th-1 cell cytokine IL-2. Interleukin-10 is known as a cytokine that suppresses T cell proliferation and cytokine response, either from Th1 or Th2 cells, triggering peripheral T cell tolerance through inhibition of CD-28.[6]

However, IL-10 showed other defenses mode, especially in the epithelial cells during infection by encouraging the natural immune response of epithelial tissue to inhibit destruction by viral and bacterial infections, hence suppress pro-inflammatory responses during inflammatory.[7] From our study seems that at low concentration, kefir generates immunorepressive effect rather than immunostimulant effect. Our result was different from study done by Vinderola (2005) who found the immunomodulatory effect of kefir. This could be due to the different research subject. Venderola used *mice model* in her study and we use human peripheral blood. It is common that *in vivo* and *in vitro* study somehow produced different result. Other factor might be due to different source of kefir. She used cow's kefir and we used goat's kefir. The source of milk from where the kefiran is produced influence greatly the complex of kefiran containing different kind of microorganism including bacteria, yeast and fungi.[3] Due to the different role of Th2 cytokine IL-10, therefore it is necessary to study further *in vivo* the immunomodulatory effect of kefir on infectious diseases, allergies, also in autoimmune diseases by using higher concentrations of kefir. This is because kefir usually consumed in 100% concentration.

CONCLUSION

Low concentration of kefir apparently modulate immune system of healthy person by shifting the

immune response towards Th-2 cells through increasing level of IL-10.

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