Expression of $CD_4^+$ T cell, Interleukin 2 and Interleukin 4 On Splenectomy Balb/c Mice Post-Exposure Salmonella typhi.

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Introduction
Splenectomy has been known as the cause of several infection due to the loosing of macrofag and other immune component like lymphocite (Kahn, 2004). Enhancing infectious by Gram negative bacteria and bacteria encapsulation likes Salmoella typhi lead to mortality of patients post splenectomy (Deodhar and Kakkar, 2004). It was reported that on 1996, 19680 patients which did splenectomy, 680 suffered infectious and 1.4% of them passed away. Salmoella typhi are facultative Gram negative anaerob bacteria which have flagella. Their pathogen characteristics used as antigen in many researches to observe the immune response (Seddon, B., and Mason, 1999). Normally, these bacteria attack intestine, payer’s patch and lymphoid pathes (Suciu-Foca, et al., 2003).

In post splenectomy, patients were vulnerable to infection and require compensation for the amount of lymphocytes to handle pathogens that invade the body. Interleukin -2 (IL-2) and IL-4 are the compensators agents involved in proliferation and development of T lymphocytes. Immune response concerning antigen are conducted by cytokines, cells, organs, and other immunity organs. Interleukine 2 (IL-2) is one of the cytokine which play a role in adaptive immunity and growth factor of T cells regulators. This cytokine support T cells proliferation especially CD4. When antigen stimulate T cells CD4, IL-2 were synthesized. Besides, IL-2 works at B cells as growth factor to induce antibody production. IL-2 also plays a key role to raise other cytokines synthesis, which is interferon gamma (IFN-γ) to activate macrofag. Macrafag activation will induce fagocytosis of pathogen bacteria and antigen. Moreover, IL-2 also increase IL-4 production which act to induce IgE production. Other influence of IL-2 are antivirus and anti bacteria (Abbas et al., 1999)

In this study, we used splenectomized balb/c mice to elucidate the effects of secondary lymphoid organs by Salmoella typhi infection. Furthermore, we investigated the effects of splenectomy on the infiltration of CD4+ T Cell and both IL-2 and IL-4 level developing in splenectomized Balb/c mice and infected by Salmoella typhi, which have a complete absence of secondary lymphoid organs.

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Materials and methods
Animals: Twelve mice (Balb/c, 12 weeks old) were used in this study. Factorial experiments (complete random design) with three replication, conducted in four groups as follows control groups, splenectomized group, S. typhi exposed group and splenectomized group followed by S. typhi exposure. Splenectomy was performed performed on a clean bench using standard techniques, use premedication of atrophiol sulfat (0.04 mg/kgBW) with xylazin (12 mg/kgBW) and Ketamine anesthesia (60 mg/kg BW). Exposure of S. typhi was performed by injection of acute doses ($10^9$ cell/ml) couple weeks after splenectomy and couple weeks after first injection. All animals were bred and maintained in our animal facility in University of Brawijaya, Indonesia, under pathogen-free conditions, and treated in accordance with the guide for animal experimentation of Ethical Committee in The care and use Animals, University of Brawijaya.

ELISA: Blood samples were collected via the apex of heart tail, and centrifuged (1500 g, 15 minutes at 4°C). Serum samples were removed. Both of IL-2 and IL-4 were measured using a commercially available ELISA quantitation.

Flow cytometry: The relative density of CD4$^+$ T cells were quantified BD FACSCalibur™ Flowcytometer.

Histology of Small Intestine
Histology technique used for analysis number of microbes penetration on small Intestine. The sections were stained with hematoxylin-eosin (HE) and then examined with light microscopy.

Statistical analysis: Data were analysed using Variance (ANOVA), using software programe SPSS for Windows Release version 13.0.

Result
Analysis of Interleukin 2 dan Interleukin 4 by ELISA Technique
The results showed that S.typhi exposure in splenectomized and nonsplenectomized mice can increase
levels of IL-2 and IL-4 significantly (p <0.05), which indicates the existence of the body's immune system at the absence of spleen and the penetration of bacteria. Additionally mice with splenectomy treatment showed the highest concentration of IL-2 and IL-4.

Interleukine 2 is one of the cytokine which play a role in immune system against antigen. The aim of IL-2 and IL-4 degree measurement was to find out the effect of Splenectomy and S.typhi invasion concerning to IL-2 as antiviral and antibacterial. Splenectomy or S.typhi exposure individually did not significantly alter the levels of IL-4 (1.44-1.84 pg/ml), whereas injection of S.typhi following splenectomy increased the level of IL-4 up to 1.8 fold (3.25 pg/ml). Level IL-2 on all treatments, splenectomy mice, S typhi exposure and splenectomy following by S typhi exposure are 1.567, 1.352 ng/ml, 1.252 ng/ml, and control (0.494 ng/ml).

**Analysis of T CD4⁺ dan CD8⁺ T Cell by Flow cytometry**

The correlation both of IL-2 and IL-4 production with CD4⁺ dan CD8⁺ T cell analysed for following the mechanism of immune respon after S. Typhi penetrated on spleenecomy mice, especillay confirmed on mesenteric lymph node. The all treatment showed that treatment does not affect significantly (p >0.05) on the relative amount of CD4⁺ T cells and CD8⁺.

**Figure 1. The CD4⁺ dan CD8⁺ T cell on Treated Mice (SI = splenectomy- S.typhi exposure ; S = splenectomy- without S.typhi exposure; I = Not splenectomy- with S.typhi exposure)**

*S.typhi* penetration in the small intestine of mice splenectomy-S.typhi exposure showed the worst penetration than other groups, indicated by the damage of intestinal tissue structure and the number of bacteria colonies in the small intestine tissue. Although, the penetration of bacteria had not able to reduce mice health.

The precentage of CD4⁺ T cell of each group treatment are, CD4⁺ T cell on control group (32.65%), splenectomy 28.24%, and splenectomy-S.typhi 35.44%, and splenectomy-paparan S.typhi T CD4⁺ 33.65%.

**Histopathology Analysis of small intestine Halus after S.typhi exposure pada**

Based on histology of mice small intestine analysis (Fig 2): S. typhi-exposure, splenectomy, and splenectomy-S.typhi exposure showed that S typhi have possibility to penetrate through small intestine membrane with various number of S.typhi. The group of splenectomy-S. typhi exposure showed the highest level microbe penetration empaired by the others group of mice, S.typhi highly penetrate on epithel of small intestine of spleenectomy- exposure mice compare with splenectomy – non exposure S typhi (Fig 2C.), indicated by the damage of intestinal tissue structure and the number of bacteria colonies in the small intestine tissue. Although, the penetration of bacteria had not able to reduce mice health. we performed sequential histopathology of small intestine tissues showed that the intestinal pathological signs both of splenectomy- with and without S.typhi exposure. and plenectomy mice start as early as 3 weeks and that various populations of leukocytes are involved in the inflammation.

**Discusston**

Splenectomy is the surgical removal of the spleen, which is an organ that is part of the lymphatic system, plays an important, though not obligatory role, in immune function, is performed under general anesthesia. The chief risk following splenectomy is overwhelming bacterial infection, with particular susceptibility to encapsulated organisms such as Salmonella typhi.

The results showed that S.typhi exposure in splenectomized and nonsplenectomized mice can increase levels of IL-2 significantly (p <0.05), which indicates the existence of the body's immune system at the absence of spleen and the penetration of bacteria. IL-2 is one of the compensators agents involved in proliferation and
development of T lymphocytes. Splenectomy or S. typhi exposure individually did not significantly alter the levels of IL-4 (1.44-1.84 pg/ml), whereas injection of S. typhi following splenectomy increased the level of IL-4 up to 1.8 fold (3.25 pg/ml). The IL-2 concentrations from each treatment were found from the highest concentration to be: mice with splenectomy was known as 1.567 ng/ml, mice with S. typhi invasion was found to be 1.352 ng/ml, and both with splenectomy and S. typhi treating of 1.252 ng/ml, respectively. Control treatment was found to be 0.494 ng/ml. Neither splenectomy nor S. typhi invasion did not alter the IL-4 concentration significantly (1.44-1.84 pg/ml). On the contrary, S. typhi post splenectomy enhanced the IL-4 concentration 1.8 times to be 3.25 pg/ml. Based on those results, it could be noted that the IL-2 and IL-4 played a role to respond the onset of bacteria and the loss of secondary lymphoid organ which important to immune system. (Rifa’i, 2000, Helmut, 2001 Chatenoud et al., 2001; Shevach, 2003 and Rifa’i, 2008) reported that there was an increasing of lymphocyte cytokines Th1 level significantly (p<0.05) in periferal region involved IL-2 dan IFNγ, also the increasing the amount T cells CD4+ and CD8+ post splenectomy. IL-2 and IFNγ play a key roles to activate macrofag and enhance the fagocytosis also stimulate production other cytokines which involve to overcome E. coli infectious at mice intestine post splenectomy (Thornton, and Shevach, 2000, Takahashi et al., 2000; Helmut et al., 2001, Malek et al., 2002, Bach, 2003).

The calculation of the number of CD4+ T cells in the mesenteric lymph nodes showed that treatment does not affect significantly (p>0.05) on the relative amount of CD4+ T cells. Splenectomy significantly depleted the relative density of bone marrow B220+ cells 32 fold down to 0.50-0.55% compare to controls (17.34%), moreover exposure of S. typhi stimulated the relative density of bone marrow CD4+ T cells up to two fold higher (0.50-0.56%) than control (0.28%). T cells, which mediate adaptive immunity, are an early and important component of lesions, and macro-phages, which play a key role in both lesion development and in mediating plaque disruption, serve to bridge innate and adaptive immune processes.

S. typhi penetration in the small intestine of mice splenectomy-S. typhi exposure showed the worst penetration than other groups, indicated by the damage of intestinal tissue structure and the number of bacteria colonies in the small intestine tissue. Although, the penetration of bacteria had not able to reduce mice health. we performed sequential histopathology of small intestine tissues showed that the intestinal pathological signs both of splenectomy- with and without S. typhi exposure, and splenectomy mice start as early as 3 weeks and that various populations of leukocytes are involved in the inflammation. Furthermore, we investigated flocytometrically the effects of splenectomy on the infiltration of CD4+ T, CD8+ T, B cells and into small intestine tissues and the relationship between mononuclear cells and inflammation of intestine developing in splenectomized mice, which have a complete absence of secondary lymphoid organs.

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Conclusion

Salmonella typhi exposure in splenectomized and non splenectomized mice can increase levels both of IL-2 and IL-4 which indicates the existence of the body's immune system in the absence of spleen and it's when exposure by bacteria.

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References


