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Shigella dysenteriae Pili Proteins as an Adhesive Molecule Can Protect Moving Solution by Using Mice Legated Ilea Loop Model

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Abstract: Shigellosis is still a big problem in developing countries. Prevention using a vaccine so far there has not be any suitable vaccine. Molecule adhesion of bacteria can serve as a basic component of the vaccine. *S. dysenteriae* sub-unit pili protein which has MW 7.9 and 48.9 kDa molecule is adhesive. Rabbit Ileal Loop Model can evaluate bacterial diarrhea. Similarly, Mice Legated Ilea Loop Model can be used to evaluate the bacterial diarrhea as well. The study are to clarify that mice which immunized with *S. dysenteriae* sub-unit pili molecular weight 7.9 and 48.9 kDa as an adhesive molecule can protect moving solution into lumen intestine and to prove that Mice Legated Ilea Loop Model can be used to show diarrhea protection. The study was conducted by protectively test with post control study design and Mice Legated Ilea Loop Model. The first group without immunization was used as a control group. The second group was immunized with *S. dysenteriae* sub-unit pili protein which has molecular weight 7.9 kDa. Third group was immunized with *S. dysenteriae* sub-unit pili protein which has MW 48.9 kDa. The last group was immunized with combination of *S. dysenteriae* sub-unit pili proteins which has molecular weight 7.9 and 48.9 kDa. Data were analized by ANOVA and Tuckey test. The significant differences in intestines weight in the control group between second, third and last group. There was no different effect in the addition of different proteins. *S. dysenteriae* sub-unit pili protein which has MW 7.9 and 48.9 kDa can be used as candidate of vaccine shigellosis and the MLIL test can be applied for studying bacterial diarrhea.

Key words: *Shigella dysenteriae*, pili, molecule-adhesive, mice ligated ilea loop, bacteria

INTRODUCTION

Shigellosis is diarrheal disease which caused by four *Shigella* sp. The disease is remains big problem in the developing country. It has been estimated one million hundred thousand people died due to shigellosis. From this data, 60% was found at up 5 years old children (WHO, 2012). *S. dysenteriae* is the most virulent and very powerful to produce diarrhea. The overall incidence of treated shigellosis was 2.1 episodes per 1,000 residents per year in all ages and 13.2/1,000/year in children under 60 months old (Von Seidlein *et al*., 2006).

Currently, no licensed vaccine targeting Shigella exists. Shigella has been a longstanding World Health Organization target for vaccine development and sharp declines in age-specific diarrhea/dysentery attack rates for this pathogen indicate that natural immunity does develop following exposure; thus, vaccination to prevent the disease should be feasible. Several vaccine candidates for Shigella are in various stages of development (Kotloff *et al*., 1999; WHO, 2012).

Shigella dysenteriae protein sub-unit pili which has MW 7.9 and 48.9 kDa molecule is adhesive can cause immune mice and will prevent come out solution from intestine to lumen by using Mice Ilea Legated Loop (MILL)

MATERIALS AND METHODS

Protectively test: The method of protectively test referred to Sumarno *et al*. (2011), Bab/c was immunized with protein sub-unit pili MW 7.9 and 48.9 kDa according to the previous study (Setyorini *et al*., 2013). Mice was divided in four groups and every grouped consists of four mice. As a control is the first group without immunization. *S. dysenteriae* protein sub-unit pili which had MW 7.9 kDa was immunized in the second group. The third group was immunized by *S. dysenteriae* protein sub-unit pili which has MW 48.9 kDa. Farther more the last group was immunized combine *S. dysenteriae* protein sub-unit pili which had MW 7.9 with 48.9 kDa. The choosen adjuvant immunogenic was ISCOM. Three times

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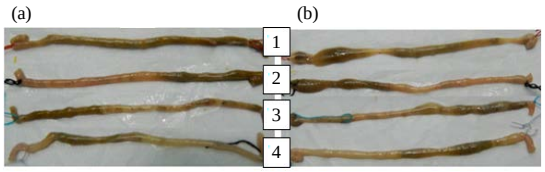

Fig. 1: The results of protectively intestine exposed with *S. dysenteriae* with various immunization used MLIL Model: a) MLIL before exposed with *S. dysenteriae* and b) MLIL after exposed with *S. dysenteriae*; 1: MLIL control; 2: MLIL immunized with protein 7.9 kDa+ISCOM; 3: MLIL immunized with protein 49.9 kDa+ISCOM; 4: MLIL combine immunized protein 7.9 kDa with 48.9 kDa+ISCOM

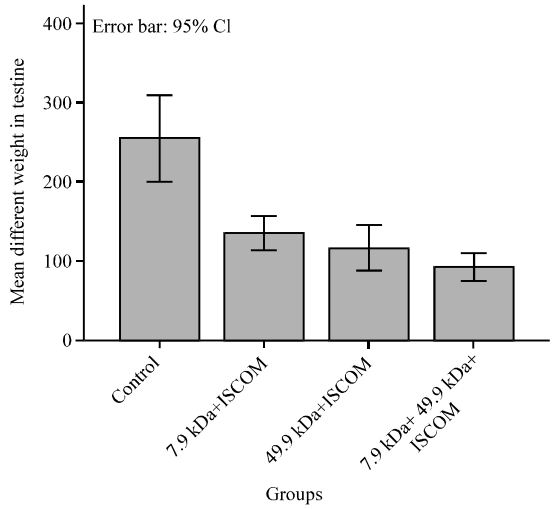

Fig. 2: The relationship between the four treatment groups weight difference MLIL before being exposed with after being exposed to *S. dysenteriae*

of immunization was given with interval 1 week. The 1 week after the last immunization all of mice was sacrificed according to protocol of ethical clearance. Abdominal cavity was opened by longitudinal incision. Intestinal part was come out and separated from the omentum. The end of intestinal part was cut at the measure over 15 cm. The end piece of cutting was legated (MLIL). Heavy MLIL weighed and after that included in the Roswell Pack Medium Institute (RPMI) 1000 mL in flash. Put in the shaker water bath and adjusted 60 times/min, temperature 370°C 4 h. Then, MLIL was removed from flash and recorded MLIL weight.

Results of research is displayed in a Fig. 1, 2 and Table 1. Data analysis use ANOVA and Tukey's test and result delivered in the form bar diagram.

Table 1: Differences in intestine weight of each sample group MLIL before being exposed with after being exposed to *S. dysenteriae*

Samples	Mean±SE	Siq.
Control	255.20±19.615	a
7.9 kDa+ISCOM	135.60±7.7820	b
49.9 kDa+ISCOM	116.60±10.297	bc
7.9 kDa+49.9 kDa+ISCOM	92.80±6.3670	bcd

RESULTS

To find out the amount of liquid in the lumen intestine by using MILL Model can be seen in Fig 1. Figure 1 shows apparently MLIL A group bigger B even though not clear. Weighing of every MLIL should be done to confirm. The result is shown in Table 1 and Fig. 2.

Table 1 and Fig. 2 show a significant difference the control group without immunization between immunization *S. dysenteriae* protein sub-unit pili which had MW 7.9 kDa+ISCOM, between immunization with *S. dysenteriae* protein sub-unit pili which had MW 7.9 kDa 49.9 kDa+ISCOM and between combine immunization with *S. dysenteriae* protein sub-unit pili MW 7.9 kDa and 49.9 kDa+ISCOM.

No significant difference were found the group immunization with *S. dysenteriae* protein sub-unit pili which had MW 7.9 kDa+ISCOM between group immunization with *S. dysenteriae* protein sub-unit pili which had MW 49.9 kDa+ISCOM and the group combine immunization with *S. dysenteriae* protein sub-unit pili which had MW 7.9 kDa+49.9 kDa+ISCOM.

DISCUSSION

The results of the research are shown in Fig. 1 and there are not clear any difference in the dimension of MLIL in each group before immunization between after immunization if the samples were exposed with the bacteria *S. dysenteriae*. To confirm MLILs sample have any difference there for must be weighed. These results are identical to our other studies (Sumarno *et al*., 2011) but different dimension and performance of MLIL will be found in the use of immune-stimulant Sumbawa fermented mare's milk and *V. cholerae* as a model (Faisal *et al*., 2010). The results different require to clarity regarding the immune-stimulant effect of fermented mare's milk Sumbawa. In addition to the examination in protective tests using MLIL also can be checked s-IgA levels, the calculation of the bacteria attached to the gut and observation of damage mucosa enterocyte (Sumarno *et al*., 2011; Setyorini *et al*., 2013).

After being confirmed by weighing of every MLIL (Fig. 1) and statistical analysis obtained in Table 1 and Fig. 2.

The conclusion *S. dysenteriae* protein sub-unit pili which has MW 7.9 and 48.9 kDa as a molecule adhesion can protect come out solution from intestine to lumen and degree of protection within *S. dysenteriae* protein sub-unit pili which has MW 7.9, 48.9 kDa and the combinatioin were not difference.

The role of the mucosal immune response is very important in driving away of intestinal microbial pathogens. Th17, IL17, IL23 available on sub-mucosa, lipocalin, β-defensin, calprotectin and s-IgA are found in the fluid of the intestinal mucosa can be known in MLIL Model (Sumarno *et al*., 2011; Abbas *et al*., 2012). MLIL Model apparently can explore research associated with the occurrence diarrhea (Everest *et al*., 1993; Knoop, 1979).

One study conducted by Zhang *et al.* (2003) using another method namely Rabbit Ileac Loop (RIL) Model to show the accumulation of fluid in the lumen of the intestine and damage of enterocyte with exposure *V. cholera* (Zhang *et al*., 2003). MLIL Model also can be shown of the accumulation of fluid in the lumen of the intestine, damage of enterocyte and bacterial colozation *S. dysenteriae* (Setyorini *et al*., 2013; Faisal *et al*., 2010).

Method of RIL is a standard method to study microbial infectious diarrhea. The method was introduced starting in 1953 after found by De and Chatterjee (1953). Up to know (from 60 years ago) is still used to determine diarrhea caused by intestinal bacterial pathogens. Broadly speaking have five procedures are: abdomen rabbit is sliced in state of anesthesia. Furthermore, after the completion slices the intestine is removed and part of the intestine 10 cm a long tied with thread. Then, intestinal tied injected with bacteria to be studied. After that the abdominal wall incision is closed with sutures and rabbit releases into the cage. For evaluating the diarrhea have to wait 6 between 8 h by doing killed the rabbit. Diarrhea process can be seen in part of intestinal tied.

At least there are three differences if we compare RIL Method between this research (MLIL). The first on MLIL Method use the degrees lower animal. The second difference in MILL animals were killed directly but on RIL Method given the opportunity to live 6 until 8 h after the abdominal incision closed. At this time the rabbit may be pain after narcotics effect lost. The third difference is MLIL *ex vivo* and *in vivo* RIL.

CONCLUSION

The conclusion of this study is the significant difference the weight of MLIL control group (without immunization) when compared with all groups of immunization. Then, within of mice immunized the weight of MLIL no significant different. After that when viewed from the ethical clearance question arises whether the MLIL Method can replace the RIL Method for the type of research that is related to the pathogenesis of pathogenic intestinal bacteria? All contributing authors declare no conflict interest.

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