International Journal of Tropical Medicine 8 (1): 19-26, 2013
ISSN: 1816-3319
© Medwell Journals, 2013

Protectivity of Adhesion Molecules Pili 49,8 kDa *Shigela dysenteriae* Conjugated with ISCOM against Bacterial Colonization and Colonic Epithelial Cells Damage in Mice

[1]Dwi Setyorini, [2]Yulian Dwi Utami, [3]Edi Widjayanto, [4]Sri Winarsih,
[4]A.S. Noorhamdani and [4]Sumarno Reto Prawiro
[1]Program Study of Magister Biomedic, [2]Program Study of Nursery,
[3]Laboratory of Clinical Pathology,
[4]Laboratory of Microbiology, Faculty of Medical,
Brawijaya University, Malang, Indonesia

Abstract: Shigellosis is a major public health problem, especially in developing countries and the disease caused by *Shigella* sp. Among four species of *Shigella*, *S. dysenteriae* is the most virulent species and is often isolated from cases of Shigellosis. Vaccination is the best effort to eradicate infectious disease that has relation to the Shigellosis. The earlier study reported that protein sub unit pili with Molecular Weight (MW) 49.8 kDa *S. dysentriae* is the hemagglutinin protein and serves as an adhesion molecule of *S. dysenteriae*. The aim of this study is to prove the ability of adhesion molecule subunit pili with MW 49.8 kDa *S. dysenteriae* to increase s-IgA that is protective against *S. dysentriae* bacterial colonization and protective against the colonic epithelial cells damage. This study was performed by using Balb/c mice divided into 4 group treatments: negative control (no immunization and no *S. dysenteriae* exposure); positive control (no immunization but the sample was exposed to bacteria); the sample immunized with 12 μg/25 μL ISCOM and exposed to *S. dysenteriae* and then the sampleMW 49.8 kDa *S. dysentriae* conjugated with 12 μg/25 μL ISCOM and exposed to *S. dysenteriae*. Immunizations were given on the day 0, 7 and 28. On the day 35, the mice were killed and the s-IgA concentrations were examined using ELISA. Colon protection was measured with Mice Ligated Ileal Loop (MLIL) Method followed with the counting of colon bacterial colonization by using bacterial colony counter after culturing on Salmonella Shigella Agar (SSA). Histopathology examination was used to identify the magnitude of colonic epithelial cell damage. The results showed that immunization used adhesion molecule subunit pili with MW 49.8 kDa *S. dysenteriae* conjugated with ISCOM could increase s-IgA concentration, reduce the colonic bacterial colonization and minimize the colon epithelial cells damage in negative control compared to positive controls and ISCOM immunization ($p<0.05$). It can be concluded, oral administration of *S. dysenteriae* pili 49.8 kDa conjugated with ISCOM can increase s-IgA protective against bacterial colonization of the colon and colonic epithelial cells damage.

Key words: Adhesion, s-IgA, colonization of *S. dysenteriae*, colonic epithelial cells damage, protein, Indonesia

INTODUCTION

Shigellosis is a major public health problem, particularly in developing countries. Approximately one million and one hundred people are expected to die by infection, 60% of which is found children under 5 years (WHO, 2009).

A survey conducted in six Asian countries (Bangladesh, China, Pakistan, Indonesia, Vietnam and Thailand) in 2000 to 2004 showed that the incidence of shigellosis in Indonesia was 3.8/1000 population per year occurring at any age and 18.6/1000 infants each year (Seidlein *et al*., 2006).

Pathogenesis of Shigellosis begins with the ability of attachment and colonization by doing adhesion molecule to mucous cell intestinal (enterocyte). The next process is intracellular invasion and destruction of cells host. Pili is one bacterial adhesion organelles (An and Friedman, 2000). The recent study reported that the protein subunit pili of *S. dysenteriae* with Molecular Weight (MW) of 49.8 kDa is a hemagglutinin protein and as *S. dysenteriae* adhesion molecule (submitted).

One of treatments for diseases caused *Shigella* sp. is by administering antibiotics but resistant has been found with some antibiotics treatment, especially *S. dysenteriae*

Corresponding Author: Sumarno Reto Prawiro, Laboratory of Microbiology, Faculty of Medical, Brawijaya University, Malang, Indonesia

that has the most common resistance among others (Ashkenazi *et al*., 2003; WHO, 2005). Therefore, prevention by vaccination is the best strategy for eradication of Shigellosis.

The main defensive mechanism of mucosal surface is secretory Immunoglobulin A (s-IgA). S-IgA of adhesion molecule subunit pili with MW 49.8 kDa *S. dysenteriae* bound to the antigen as an adhesion molecule therefore, prevents adhesion to the surface of the mucosa. Subsequently it is excreted via peristalsis or mucocilliar movement called as immune exclusion (Ogra *et al*., 2001). Whether there is any increase of s-IgA by induction of adhesion molecule subunit pili with MW of 49.8 kDa should be clarified. The purpose of this study was to prove whether the *S. dysenteriae* adhesion molecule subunit pili with MW 49.8 kDa can induce and increase s-IgA and there fore becomes protective against *S. dysenteriae* bacterial colonization and colonic epithelial cell damage.

MATERIALS AND METHODS

This study was an experimental research laboratory and used as its post-test research design. The subjects used were Balb/C male mice that were divided into 4 groups.

Culture *Shigella* sp.: Bacteria *S. dysenteriae* was obtained from Surabaya Regional Health Laboratories East Java Indonesia and developed in TCG medium to enhance the growth of bacterial pili. This medium contained 0.02% thioproline, 0.3% $NaHCO_3$, 0.1% mono sodium l-glutamate, 1% bactotryptone, 0.2% yeast extract, 0.5% NaCl, 2% bacto agar and 1 mM amino-ethyl ether-N, N, N, N-tetra acetic acid (EGTA) (Ehara *et al*., 1987).

Method of isolation of *S. dysentriae* pili: Isolation of *S. dysenteriae* pili referred to the research done by Sumarno *et al*. (2012) with modification. Bacteria pili was cut by using pili bacterial cutter and the cutting was carried out for 30 sec at a speed 5000 rpm while the second to fourth cutting used the same speed. The isolation of pili fraction by centrifugation of cutting result was done at 12,000 rpm using a temperature 4°C. The supernatant containing the bacterial pili were stored at a temperature of 4°C.

Method of isolation of protein sub unit pili with molecular weight 49.8 kDa *S. dysenteriae*: Research method referred to Sumarno *et al*. (2011). The results of pili collection was carried out by SDS-PAGE Method. The results of gel electrophoresis took the form of a straight cut at the desired molecular weight. Then, the pieces were cut perpendicularly so each piece will contain three protein bands. The results of the above pieces of band were collected and then inserted into the tube of dialysis membrane by using electrophoresis running fluid buffer. Elusion from the band used a horizontal electrophoresis apparatus at 125 mV power for 25 min. Then, the results of elusion underwent dialysis with PBS pH 7.4 buffer fluid as much as 2 L for 2×24 h. Fluid of dialysis was replaced three times and fluid in membrane of dialysis results from elution of SDS-PAGE band was ready for hemagglutination testing.

Sodium Dodecyl Sulfate Gel Electrophoresis Polyacrylamid (SDS-PAGE): Monitoring was done by using the Molecular Weight SDS-PAGE Method by Laemmli (1970). Protein samples were heated at 100°C for 5 min in a buffer solution containing 5 mM Tris HCl pH 6.8, 5% 2-mercapto ethanol, 2.5% w/v sodium dodecyl sulfate, 10% v/v glyserol by using color tracker Bromophenol blue. Then, 12.5% mini slab gel with a 4% gel tracking was selected. Electric voltage used was 120 mV. As the color of the material Coomassie brilliant blue and sigma standard fermentas marker were used.

Immunization: Immunizations were given on day 0, 7 and 28 orally (under the guidance of ABISCO of ISCONOVA). Group I as negative control didn't obtain immunization and wasn't exposed to bacteria; group II as positive control did not obtain immunization but was exposed to bacteria, group III obtained immunization with ISCOM and was exposed to bacteria and group IV: obtained immunization with adhesion molecule subunit pili MW 49.8 kDa *S. dysenteriae* + ISCOM and was exposed to bacteria.

Preparation of mucus: Preparation of mucus was carried out as follows: intestinal pieces were washed with cold PBS. Then, the intestine was opened so that the visible part of the small intestine mucosa exposed. Layer of mucus was collected by scraping longitudinally with spatel and placed in tubes containing sterile PBS and protease inhibitors. The suspension was shaken then centrifuged at 12,000 rpm in 40°C for 10 min. The supernatant was taken to perform purification of s-IgA. The supernatant was resuspended with PBS after that dialysis was performed with the use of PBS and used as a sample for examination of s-IgA by ELISA Method.

S-IgA examination by ELISA Method: S-IgA examination was done by ELISA method with the use of anti mouse s-IgA ELISA kit from NovaTEinBio. The parameter measured were the levels of s-IgA in the intestinal mucous of mice. The kit was removed from temperature 2-8°C and left at room temperature for 30 min

before the use. Sample 50 μL diluent was put into first well as a blank followed by the rest well. After that 50 μL standard sample was put into second to seventh well. Then, 100 μL HRP-conjugated antibody was added to each well closed with allumonium foil and incubated at 37°C for 1 h by using agitation. Subsequently sample was washed by washing solution 5 times. Then, 50 μL chromogenic A substrate and chromogenic B substrate was added into each well. After that it was put in shacker incubator incubated at 37°C for 15 min and closed with aluminum foil to prevent of destruction from light. Then, 50 μL stop solution was added and it was immediately observed by using ELISA reader with OD at λ = 450 nm (NovaTEinBio Elisa kit Instruction).

Protectivity test: Protectivity test used was according to earlier study (MLIL/Mice Ligated Illeal Loop Model). Colons of mice in this study were taken along the end of the small intestine to the rectum and cut into 2 parts, 4 cm at proximal and 4 cm at distal part. Then, both ends of each were tied with twine. Then of 10^6 mL^{-1} *S. dysenteriae* was injected after that it was inserted into 200 mL Roswell Pack Medium Institute (RPMI) and was stirred with temperature 37°C. Time of exposure to the bacteria was 4 h (Sumarno *et al*., 2011).

Calculation of bacterial colonization of the colon: Colon which had been exposed to the bacteria *S. dysenteriae* for 4 h then was opened, feces were removed and rinsed with sterile PBS. Then, the colon was cut 10×10 mm and was homogenized with a Potter homogenizer by using sterile PBS at the same volume. Omogenate was taken 100 mL and planted on solid medium SSA. SSA medium was incubated for 18-24 h at 37°C. The grown colonies were identified and those characterized with *S. dysenteriae* were calculated by colony counter.

Histopathological examination and extensive colonic damage: Histopathological examination and extensive colonic damage was carried out in the anatomical laboratory medical faculty of Brawijaya University Malang by using methods commonly used. In broad outline it can be explained: colon that had been exposed to the bacteria *S. dysenteriae* for 4 h then was put in formalin and left to soak in it for 24 h. Tissue was cut into pieces with thickness of approximately 2-3 mm (cross-sectional sample with a colon was cut into three parts). Kekaset was added and process continued by using the tools/machines tex tissue processor. Furthermore, the paraffin tissue was blocked and cut with a microtome with thickness of 3-5 μ. Then, deparaffinization and coloration of HE were carried out. Further dehydration and purification processes (clearing) was performed. Then, it was followed by the mounting with entelan and deckglass.

After histopathological preparation of colon and HE staining, photographing and scanning were carried out with OLIVIA Software (Olympus Imaging Viewer for Application) and then extensive damage was measured. Extent of damage was calculated based on the average of the three parts of the piece.

Data analysis: Statistical analysis used ANOVA. Research was significant when $p<0.05$ was achieved. Post hoc analysis was then performed with the Tukey test if there were significant differences. If the data didn't meet the requirements of normality and homogeneity based on Kruskall Wallis test then it was tested further by Mann Whitney.

RESULTS

Isolation of protein sub unit pili with molecular weight 49.8 kDa *S. dysenteri*: For vaccine material, protein sub unit pili with moleculer weight 49.8 kDa *S. dysentriae* was isolated in the same way with methods by Prabowo (2010). The result of such proteins is shown in Fig. 1. To make sure the isolatedd one was truly protien sub unit

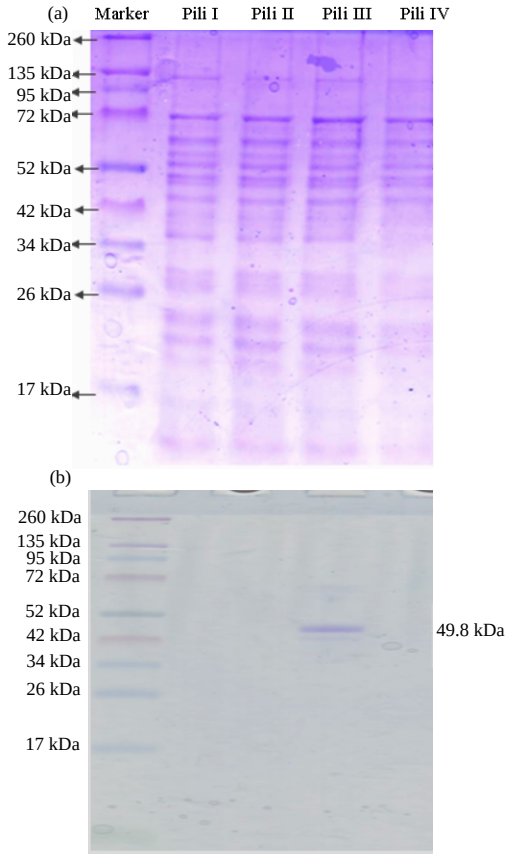

Fig. 1: Fractionation profile of pili *S. dysenteriae*

pili with MW 49.8 kDa *S. dysentriae*, the isolated protein underwent electro elution again with SDS PAGE (Fig. 1b).

The results of measurement s-IgA: Group I: control (not immunized and are not exposed to bacteria), group II: control (not immunized but exposed to bacteria), group III: ISCO Mimmunization, bacteria exposed; group IV: 49.8 kDa + ISCOM immunization, bacteria exposed.

Mann Whitney test results showed no difference between group I, namely a control group that was not immunized and by group III that was immunized with ISCOM immunization ($p = 0.754$).

In contrast there is a significant difference between group I (control) and group IV that obtained pili immunization 49.8 kDa protein + ISCOM ($p = 0.009$) and between group III (ISCOM immunization) and group IV with pili 49.8 kDa protein immunization + ISCOM ($p = 0.028$). It proves that immunization of 49.8 kDa protein adhesin + ISCOM can increase levels of s-IgA intestinal mucous (Table 1).

The results of colony counting of *S. dysenteriae* bacteria in the mice colo: The next process was counting the bacterial colonies that had been cultured on SSA medium and incubated for 18-24 h at 37°C. The grown colonies were identified and only bacteri characterized with *S. dysenteriae* were calculated by colony counter. Results in Fig. 2 appears in Fig. 3 which shows the difference difference of *S. dysenteriae* colony growth on *e*SSA medium for all 4 treatment groups (colony counting). Group I: obtain no immunization and not exposed to the bacteria, no presence of bacterial colonies growth, group II: obtain no immunization but exposed to bacteria, presence of many bacterial growths, group III obtain ISCOM immunization alone and exposed to the bacteria, presence of bacterial growth lower than group II, group IV obtain pili immunization 49.8 kDa protein and ISCOM and exposed to bacteria, presence of bacterial growth less than group II and III. Bacterial colonization is calculated only based on colonies characterized with *S. dysenteriae*.

Kruskall Wallis test results showed significance value of 0.001. Since, the significance value is $<\alpha$ ($0.001<0.05$) it can be stated that there were significant differences between treatments.

Table 1: The difference in levels of s-IgA

No.	Groups	Mean±SD (μg mL^{-1})
1	I	1.235±0.022^{a}
2	II	1.219±0.020^{a}
3	III	1.289±0.174^{a}
4	IV	1.629±0.303^{b}

Different letter indicates significantly different while the same letter means not significantly different with $p<0.05$

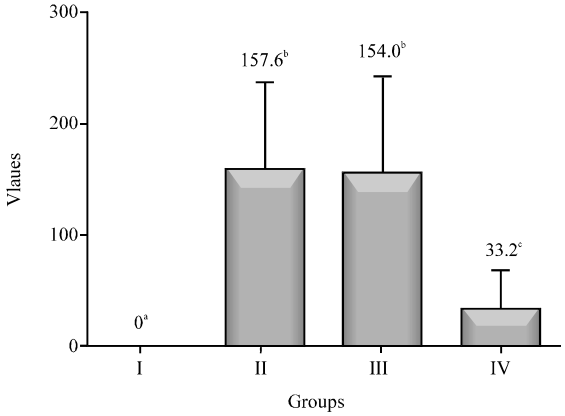

Fig. 2: The number of becterial colonization in the colon

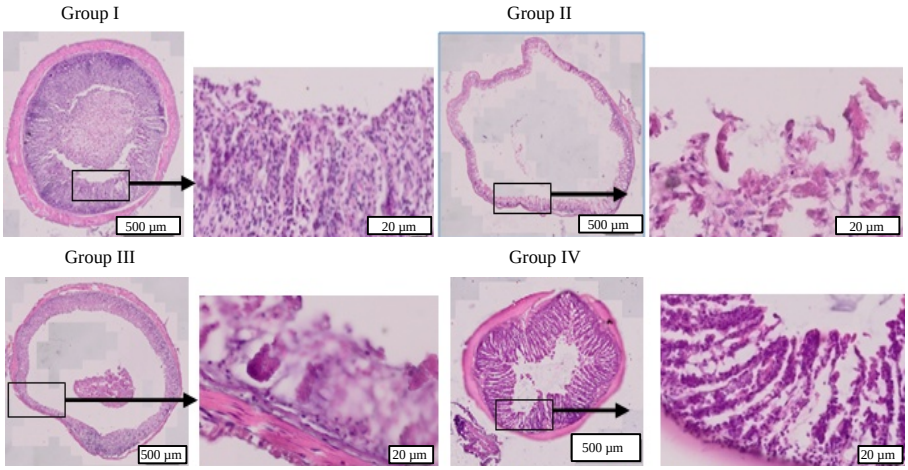

Fig. 3: Histopathology the colon with HE staining (scale: 500 μm left; right 20 μm)

Table 2: The number of *S. dysenteriae* colonies in cultured colon

No.	Groups	Mean±SD (μg mL^{-1})
1	I	0.0±0.000^{a}
2	II	157.6±79.25^{a}
3	III	154.0±87.85^{a}
4	IV	33.2±34.79^{b}

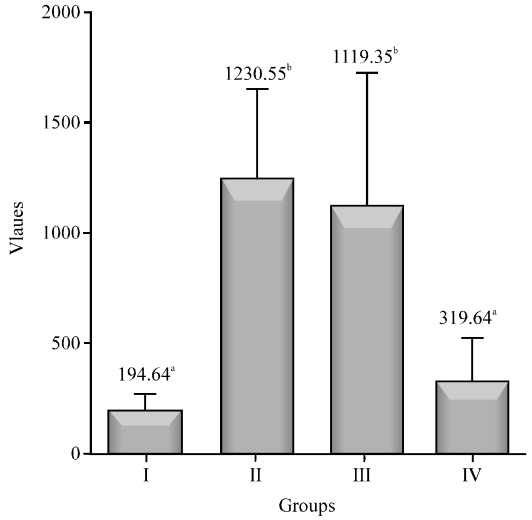

Fig. 4: Area damage of colonic epithelial cells (μm)

Mann Whitney test showed difference between the I-IV groups ($p = 0.005<\alpha$) and there were also differences between groups II-IV ($p = 0.016<\alpha$). In contrast there was no difference between group II and III ($p = 0.916<\alpha$) (code letters in Table 2).

Colonic epithelial cell damage: Figure 4 shows, area damage of colonic epithelial cells (μm). Four treatment demostrated of the differences histopathologic picture. After histopathologic preparation of colon and HE staining, photographing and scanning were carried out with the use of OLIVIA Software (Olympus Imaging Viewer for Application) and then extensive damage was measured. Group I (negative control) showed relatively intact epithelial cells, group II (positive control), it appears showed extensive epithelial cell damage, group III (ISCOM immunization), showed epithelial cell damage less than group II and d) group IV (49.8 kDa + ISCOM immunization) showed minimum epithelial cell damage.

ANOVA test results showed that the obtained value of F count equal was 9.262 with a significance value of 0.001. Since, the sig value was $<\alpha$ ($0.001<0.05$), it can be stated that there was a significant effect between treatments.

Tukey HSD test results showed difference between groups I-III and difference between groups II-IV ($p = 0.010$, $p = 0.025$). In contrast there was no difference between group II and III ($p = 0.969$) and no difference between group I and IV ($p = 0.957$). Differences between groups can be seen on the letter code (Table 3).

Table 3: Extensive damage of colon epithelial cell

No.	Groups	Mean±SD
1	I	194.64±93.160^{a}
2	II	1230.55±423.35^{b}
3	III	1119.35±614.03^{b}
4	IV	319.64±226.85^{a}

DISCUSSION

Isolation of protein sub unit pili with molecular weight 49.8 kDa *S. dysenteriae*: Protein sub unit pili with MW 49.8 kDa *S. dysenteriae* singgle band was depicted in Fig. 1b. This singgle band protein was the result of electroelution method from protein sub unit pili *S. dysentriae* (Fig. 1a). This proteins were isolated by using PBC and the profile in SDS-PAGE were different with the earlier study (Sumarno *et al*., 2011, 2012). Differences from the results require a more in-depth research studies.

Ability of 49.8 kDa protein in inducing s-IgA: IgA plasma cells that had been induced and stimulated by antigen in the germinal center will cause an interaction between B cells, follicular dendritic cells that present antigen to local CD4 T cells and facilitates B cell proliferation, Class Switch Recombinant (CSR), somatic hypermutation and affinity maturation required for efficient humoral immune response (Brandtzaeg, 2010).

The repeated exposure of protein antigen will produce antibodies with increased affinity to ward the antigen. This process is called affinity maturation it can cause the production of antibodies with increased capacity for binding and neutralization of microbes and their toxins (Abbas and Lichtman, 2011).

Based on the above studies, it can be assumed that there was increased levels of s-IgA intestinal mucosa because of the exposure to protein antigen. Protein that act as an antigens in this study was a protein adhesion subunit pili with MW 49.8 kDa *S. dysenteriae* which was given and repeated (on days 0, 7 and 28). Repeated exposure of protein antigen will produce antibodies with increased affinity for the antigen (affinity maturation) it can cause the production of antibodies with increased capacity for binding and neutralization of microbes and their toxins.

The explanation of s-IgA prominence in the external fluid described lies on the fact that there were more lymphocytes in the lamina propia, then transported across the epithelial layer to function in the external fluid (Corthesy, 2007).

Protectifity of induction of adhesion protein 49.8 kDa *S. dysenteriae* against bacterial colonization in the colons: Pathogenesis of Shigella infection begins with the ability of bacteria to mucosal cell cause adhesion to the mucous surface of host cell. Pili/fimbria is one of bacterial adhesion organelles (An and Friedman, 2000). In other Enterophatogenic bacterial adhesion occurs in the small intestine but in Shigella adhesion occurs in colon and rectal epithelium (Sudha *et al*., 2001). Shigella adhesion in colon cells host makes these bacteria are able to prevent colonization and clearance of bowel movements. The role of specific attachment to the cell host is played by specific adhesion of FimH at the pili tip structure (Proft and Baker, 2009).

Earlier research conducted by Prabowo proved that adhesion protein 49.8 kDa *S. dysenteriae* is a protein that can inhibit *S. dysenteriae* adhesion in enterocytes cell (submitted).

Earlier exposure stated that the immunization of 49.8 kDa protein adhesin + ISCOM proved to have the highest levels of s-IgA and significantly different compared to the positive control and ISCOM immunization alone (Table 1 and Fig. 5). S-IgA protects body against potential pathogens preventing the adhesion and colonization of pathogens in the host cell. S-IgA also can work as an opsonin because neutrophils, monocytes and macrophages have receptors for Fcα-R so it can enhance the effects of bacteriolitic complement and neutralize toxins (Abbas and Lichtman, 2011).

That s-IgA can inhibit the adhesion of Shigella has been demonstrated by Willer *et al.* (2004) who conducted research on the effects of breastfeeding protein on colonization of *S. dysenteriae*, *S. flexneri* and *S. sonnei*.

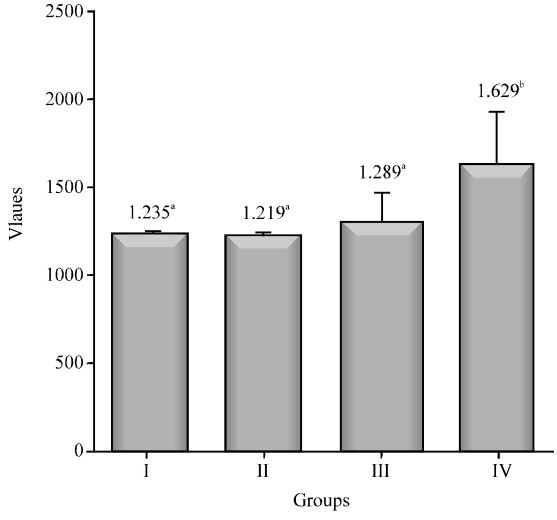

Fig. 5: Level of s-IgA in each group of mice intenstine

n and affinity chromatography. Then, the adhesion and invasion test in cell culture was conducted on milk protein that was fractionated by gel filtration and affinity chromatography. The results suggest that the adhesion and invasion of the three species of Shigella can be inhibited by s-IgA, lactoferin and free secretory component in low concentrations. The study also proved that the protein binds to the surface of the milk and the protein whole-cell bacteria.

The results of this study indicate that immunization of adhesin protein subunit pili with MW 49.8 kDa *S. dysenteriae* + ISCOM was able to reduce the number of bacteria in the colon than compared to group II (positive control) and compared to group III (ISCOM immunization) (Fig. 6, Table 2 and Fig. 2). This suggests that 49.8 kDa + ISCOM can inhibit the adhesion and invasion in colonic *S. dysenteriae*. This is consistent with several studies above stating that pili plays an important role in the adhesion of Shigella to the target cells. With the induction of protein pili 49.8 kDa *S. dysenteriae* an adhesion molecule antibodies specific to *S. dysenteriae* s-IgA will be formed. At the time of exposure to *S. dysenteriae* bacteria, the formed s-IgA removes out into the lumen of the colon and will bind *S. dysenteriae* that so it may hinder adhesion and invasion of *S. dysentriae* into the colon. However, group IV (immunization of 49.8 kDa *S. dysentriae*+ISCOM) differed significantly from group I (negative control). This suggests that along with the increase in s-IgA apparently bacteria still can invade the colonic although, the result is lower than in the positive control group. There may be factors other from other bacterial adhesion than pili such as Outer Membrane Proteins (OMP) so that the bacteria can still invade the colon.

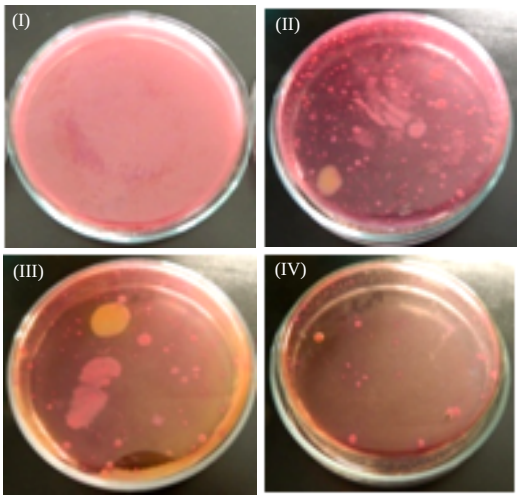

Fig. 6: Area damage of colonic epithelial cells (μm)

Protectifity of induction of an adhesion protein 49.8 kDa *S. dysenteriae* on colonic epithelial cell damage: Inflammatory lesions in Shigellosis occur in the upper rectum and distal colon. Histopathological studies stated that the lesion was found in lymphoid follicles it proved that the FAE is the main entrance of Shigella. In studies with rabbit ligated loops, *S. flexnery* entered M cell after a short infection period (2-8 h) which caused an increase in M cell size due to the accumulation of mononuclear cells. The process was accompanied by an extensive cellular damage (Perdomo *et al*., 1994; Sansonetti and Phalipon, 1999; Corr *et al*., 2007).

Research by Boullier *et al.* (2009) on the protection mechanisms mediated by Shigella LPS-specific s-IgA, used rabbit ligated intestinal loops. Containing Peyer's patches with the one given with bacteria, s-IgA, s-IgA and immune complex *S. flexneri.* After 8 h location of bacteria, s-IgA and s-IgA immune complexes *S. flexnery* were observed by immune histochemistry and confocal microscopy imaging. The results showed that in relation to the provision of anti-LPS s-IgA Shigella, mainly through immune exclusion, inflammatory prevention induced by Shigella was responsible for the damage of intestinal barrier. Besides working in the lumen, the small proportion of *S. flexnery* s-IgA immune complex indicated entry into Peyer patches and it was internalized by dendritic cells in sub-epithelial area. Local inflammation was analyzed RT-PCR quantitative by using new design primers for rabbit pro-and anti-inflammatory mediator gene. In Peyer patches that showed the immune complex, the up-regulation of the expression of pro-inflammatory genes was limited, including TNF-α, IL-6, Cox-2 and IFN-γ that was consistent with protection against intestinal tissue In contrast in peyer patches that just showed Shigella course there was a high expression of the inflammatory mediator-mediattor indicating that neutralizing s-IgA lowered pro-inflammatory response of Shigella. These results suggest that in the form of an immune complex, s-IgA guaranteed immune exclusion and neutralized translocation of bacteria as well as protecting the integrity of the intestinal barrier by preventing inflammation induced by bacteria (Boullier *et al*., 2009).

Mathias and Corthesy (2011) also conducted research on the effects of s-IgA on defense intestinal epithelial cell integrity. By using bacterial *S. flexnery* in Caco-2 cells, s-IgA was derived from hybridoma-isolated in the form of pIgAC5 specific for *S. flexnery* serotype 5a LPS whereas the bacteria used was *S. flexnery* M90TGFP which was virulent strains. Damage in tight junction was viewed by using Laser Scanning Confocal Microscopy (LSCM). The results indicated that s-IgA may neutralize the bacteria that cause tigh junction defense so that, the integrity of intestinal epithelial cells remains intact and this does not happen to IgG.

This study indicated that through immunization of adhesin proteins pili 49.8 kDa *S. dysenteriae* there was minimal colonic epithelial cell damage compared to the positive control group and the group III (ISCOM immunization) whereas there was no difference between group II (positive control) and the group III (ISCOM immunization) (Fig. 3 and 6, Table 3). These results are consistent with elevated levels of s-IgA on the group with the administration by of immunization with adhesin protein pili 49.8 kDa *S. dysenteriae*.

Binding of antigen by s-IgA on mucosa can be found in 3 places, namely: the lumen, s-IgA will bind antigen on the surface of the lumen and will be removed (immune exclusion) on intracellular enterocytes when antigens enter the cell and on lamina propia which produced antibodies that will bind/neutralize incoming antigen (Abbas and Lichtman, 2011).

This study shows that s-IgA may work on intracellular enterocytes so, the destruction of epithelial cells/intestinal mucosa can be minimized/prevented.

No differences between group 1 (negative control) and group IV (immunization of protein pili 49.8 kDa *S. dysenteriae* + ISCOM) found from this study. Considering that group it was not induced with bacterial at all, however there was damage to the epithelial cells despite its minimal amounts. It could be caused by some uncontrollable factors including the condition of the intestinal mucosa of mice before treatment. Also, during treatment immunization, the mice in group I (negative control) were raised at the same time with the immunization treatment group so, during the maintenance period there was the possibility of intestinal mucosal damage by other causes. It could not be controlled by the researcher as well as conditions in an enclosure that was not sterile thus, allowing the digestive tract infection due to poor hygiene. Those things are a weakness of this study that functions as the suggestions for future research.

CONCLUSION

From the results of research and presented discussion it can be concluded that the oral induction of adhesion molecule subunit pili 49.8 kDa *S. dysenteriae* can increase intestinal s-IgA, inhibit bacterial colonization in colon and prevent damage in epithelial cells of colon.

ACKNOWLEDGEMENT

This study was supported by Indonesian Ministry of Health under the scheme of IPTEKDOK.

REFFERENCES

Abbas, A.K. and A.H. Lichtman, 2011. Basic Immunology: Functions and Disorders of the Immun System. 3rd Edn., Sunders Elsevier, Philadelphia, PA.

An, Y.H. and R.J. Friedman, 2000. Handbook of Bacterial Adhesion: Principles, Methods and Applications. Humana Press, Totowa, New Jersey, Pages: 644.

Ashkenazi, S., I. Levy, V. Kazaronovski and Z. Samra, 2003. Growing antimicrobial resistance of Shigella isolates. J. Antimicrob. Chemother., 51: 427-429.

Boullier, S., M. Tanguy, K. A. Kadaoui, C. Caubet, P. Sansonetti, B. Corthesy and A. Phalipon, 2009. Secretory IgA-mediated neutralization of *Shigella flexneri* prevents intestinal tissue destruction by down-regulating inflammatory circuits. J. Immunol., 183: 5879-5885.

Brandtzaeg, P., 2010. Update on mucosal immunoglobulin A in gastrointestinal disease. Curr. Opin. Gastroenterol., 26: 554-563.

Corr, S.C., C.C.G.M. Gahan and C. Hill, 2007. M-cells: Origin, morphologyand role in mucosal immunityand microbial pathogenesis. FEMS Immunol. Med. Microbiol., 52: 2-12.

Corthesy, B., 2007. Roundtrip ticket for secretory IgA: Role in mucosal homeostasis. J. Immunol., 178: 27-32.

Ehara, M., M. Ishibashi, Y. Ichinose, M. Iwanaga, S. Shimotori and T. Naito, 1987. Purification and partial characterization of fimbriae of vibrio cholerae O1. Vaccine, 5: 283-288.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227: 680-685.

Mathias, A. and B. Corthesy, 2011. Anti inflamatory properties of s-IgA on intestinal epithelial cells during infection by *Shigella flexnery*. http://www.immun ologyresearch.ch/files/ial-poster-mathias-ssai-2011.pdf.

Ogra, P.L., H. Faden and R.C. Welliver, 2001. Vaccination strategies for mucosal immune responses. Clin. Microbiol. Rev., 14: 430-445.

Perdomo, O. J., J. M. Cavaillon, M. Huerre, H. Ohayon, P. Gounon and P.J. Sansonetti, 1994. Acute inflammation causes epithelial invasion and mucosal destruction in experimental *Shigellosis*. J. Exp. Med., 180: 1307-1319.

Proft, T. and E.N. Baker, 2009. Pili in Gram-negative and Gram-positive bacteria-structure assembly and their role in dissease. Cell Mol. Life Sci., 66: 613-635.

Sansonetti, P.J., and A. Phalipon, 1999. M-cells as ports of entry for enteroinvasive pathogens: Mechanisms of interaction, consequences for the disease process. Semin. Immunol., 11: 193-203.

Seidlein, V., D.R. Kim, M. Ali, H. Lee, X. Wang *et al*., 2006. Multicentre study of Shigella diarrhoea in six Asian countries: Disease Burden. Clin. Manifestations Microbiol. PLoS Med., 3: e353-e353.

Sumarno, R.P., A. Susanto, G. Ismanoe and S. Winarsih, 2011. Combinations of protein Sub-unit PILI 37.8 KDA *V. cholerae* with cholera toxin Sub-unit B *V. cholerae* can protect come out of the solution in the intestinal mice. J. Pharm. Biomed. Sci., 1: 154-160.

Sumarno, U. Yanuhar, S. Winarsih, S. Islam and S. Santoso, 2012. Detection of molecule adhesion sub-unit pili 48 kDa *Salmonella Typhi* by immuno cytochemistry method using sera patients suffering from typhoid fever. J. Basic. Applied Scient. Res., 2: 8527-8532.

WHO, 2005. Guidelines for the Control of Shigellosis, Including Epidemics due to Shigella Dysenteriae 1. WHO, Geneva, Switzerland, ISBN-10: 9241592330.

WHO, 2009. Diarrhoeal dissease. Initiative for Vaccine Research (IVR), WHO, Geneva, Switzerland. http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index.html.

Willer, E.M., R.L. Lima and L.G. Giugliano, 2004. *In vitro* adhesion and invasion inhibition of *Shigella dysenteriae*, *Shigella flexneri* and *Shigella sonnei* clinical strains by human milk proteins. BMC Microbiol., Vol. 4. 10.1186/1471-2180-4-18.