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Intranasal immunization with 54 kDa hemagglutinin pili protein of Streptococcus pneumonia increases mucosal and systemic antibodies

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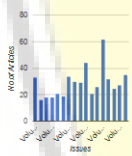

Editorial Board

Intranasal immunization with 54 kDa hemagglutinin pili protein of *Streptococcus pneumonia* increases mucosal and systemic antibodies

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Abstract:

Streptococcus pneumoniae (Klein, 1884) can cause disease with high morbidity and mortality in children under 2 yr of age, parents, and individuals with low immunity. Amount one of the diseases that can be caused by these bacteria is pneumonia. To prevent the spread of pneumonia, intranasal immunization has been developed in several studies because of its ability to improve mucosal and systemic immune responses. To prevent the attachment of bacteria to the epithelial surface of the respiratory tract, it would be more effective if intranasal immunization uses a vaccine from bacterial pili protein. The purpose of this study was to conducted to determine the ability of *S. pneumoniae* bacterial pili protein with a molecular weight of 54 kDa in increasing the concentration of mucosal and systemic antibodies through intranasal administration. Setting and design in this study using pure 54 kDa pili protein was used as an antigen to immunize Wistar mice. Mucosal antibodies were identified by the presence of sIgA in nasal washings and systemic antibodies determined from serum IgA and IgG. Statistical analysis used ANOVA. Mice immunized with combination adjuvant–antigens had higher levels of sIgA, IgA, and IgG than other groups. ANOVA statistical tests showed significant differences in sIgA and IgA levels between rats immunized with antigen–adjuvants and other groups. However, there is no significant difference from serum IgG. This study showed an intranasal immunization of 54 kDa hemagglutinin pili protein *S. pneumoniae* increased the concentration of sIgA, serum IgA and IgG.

Keywords: Antigenicity, mucosal vaccination, virulence factors, IgG, sIgA

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Introduction

Streptococcus pneumoniae (Klein, 1884) colonizes is a Gram–positive coccus that colonizes the human airways including the nasopharynx. This bacterium can cause several diseases, namely otitis media, meningitis, sepsis and pneumonia [(1)]. Pneumonia caused by these bacteria is known as pneumococcus. Pneumococcus is still one of the health problems in the world because it has high morbidity and mortality. An estimated 12 000 to 28 000 deaths from thepneumococcal disease occur in this region every year [(2)]. This bacterium has various virulence factors that have antiphagocytic functions, adhesion factors, invasion, iron transport, protection against oxidative stress, and biofilm formation. Amount one virulence factor that has adhesion function is pili [(3)]. The amount one of the pili proteins in *S. pneumoniae* which has hemagglutinin and antigenicity properties is a pili protein with a molecular weight (MW) of 54 kDa [(4)], for the development of vaccines, especially mucosal vaccinations, this protein has the potential to be used. Besides being able to induce humoral immune responses, mucosal vaccination can also induce cellular immune responses. This vaccine has several advantages including safety, ease of vaccination, economic production, induction of mucosal immune responses, and improved memory of induction of B–cells and T–cells. Importantly, the characteristic induction of the mucosal immune response depends on the vaccine delivery route chosen. For example, nasal mucosa [(5)]. Nasal mucosa is a suitable place for vaccines against respiratory infections not only because the nasal cavity is the entry point for respiratory pathogens, but also because of the immune system of the respiratory tract mucosa by *nasopharyngeal lymphoid tissue* (NALT). Intranasal immunization can prevent infection by pathogenic bacteria in the respiratory tract due to sIgA produced in the upper and lower

respiratory tract [6, 7]. Based on this background, the researchers wanted to find out the ability of the 54 kDa *S. pneumoniae* hemagglutinin protein given intranasally to increase mucosal and systemic antibodies.

Materials and methods

Ethical clearance:

The protocol of this study was approved by the Ethics Commission of the Faculty of Medicine, University of Jember with No. 698 / H.25.1.11 / KE.2015.

Isolation and purification of pili protein:

S. pneumoniae used in this study were obtained from the Central Laboratory of Health Laboratory in Surabaya, East Java, Indonesia, which was then breeding on blood agar media (BAP). Bacteria that grow on blood agar are then replanted in TCG medium to enrich pili growth. In bacteria that have been harvested, TCA is added and then homogenized and rotated using a centrifuge. Pellets obtained through the cutting process up to four times. The results of the cutting are centrifuged again, and the supernatant is taken. Separation of proteins in pili, electrophoresis was carried out using polyacrylamide gel. Its molecular weight calculates the protein that appears in the gel. The dominant molecular weight is propagated and cut for further purification using the elution and dialysis methods [8, 9].

Immunization:

S. pneumoniae used in this study were obtained from the Central Laboratory of Health Laboratory in Surabaya, The Wistar rat used in this study was 12 wk old to 16 wk old. The rats were divided into four treatment groups, each group consisting of seven mice. Rats in the control group mice were only induced with PBS 40 µL; group 1 was given 40 µL PBS and adjuvant (2 µg CTB); group 2, mice were immunized with 20 µg of *S. pneumoniae* 54 kDa pili protein in 40 µL PBS; while group 3 was immunized with a combination of 40 µL PBS, 20 µg Ag and 2 µg CT. All treatments were carried out intranasally on (0, 7, 14) d with a volume of 20 µL for each nostril. A nasal washing and isolation of nasopharyngeal tissue are done 1 wk after the last immunization. [10, 11].

Sample isolation:

Sampling was carried out after the mice were terminated by decapitation. Nasal rinses are taken by inserting 1 mL to 2 mL of sterile saline solution through the trachea and then collecting the liquid coming out of the nostrils with a sterile microtube. Nasopharyngeal tissue isolation is done by lifting the lower jaw after the scalp is cleaned up [12].

Antibody measurement:

In this study, ELISA was used to measure s–IgA, IgA, and IgG levels. The color expression seen on the microplate was measured using an ELISA reader at a wavelength of 405 nm [10, 11].

Statistical analysis:

Data analysis used in this study is one way–ANOVA with p–value < 0.05.

Results

S. pneumoniae has been cultured on biphasic medium (TCG–BHI) enriched with 5 % sheep blood. After incubated for 2 × 24 h, the bacteria harvested and the pili protein cut by bacterial pili cutter and repeated until the color of the supernatant as the same as PBS. To determine the profile of pili proteins performed by SDS–PAGE using the Coomassie blue staining. The results of SDS–PAGE can detect dominant pili proteins having an MW of (67, 54, 25, 11) kDa (fig 1). The purification process of the 54 kDa pili protein which is an antigen to immunize mice is carried out by electroelution and dialysis methods.

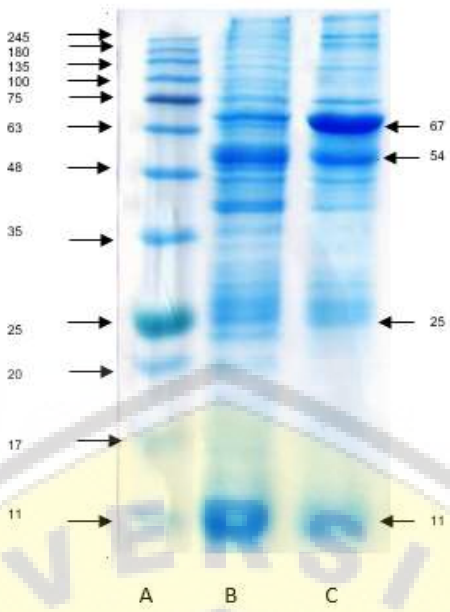
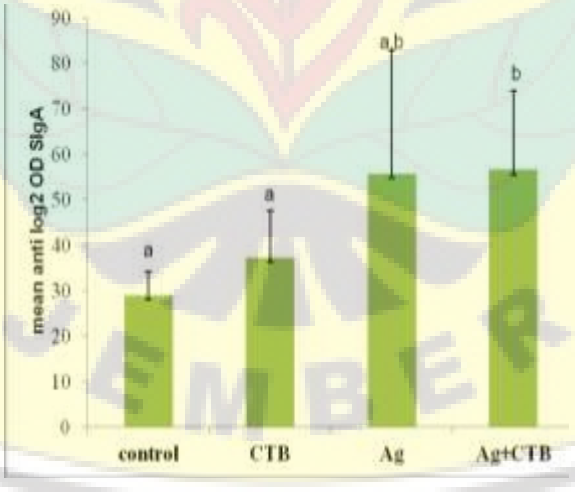


Figure 1: SDS–PAGE–profile molecular weight (kDa) pili proteins of *Streptococcus pneumoniae*. (A) protein marker (B) whole bacteria (C) pili

Nasal flushing was taken after the Wistar Rat was induced three times on (0, 7, 14) d. The next stage of this research measure the concentration of sIgA of nasal wash, IgA dan IgG serum. The ELISA test results showed that s–IgA of nasal washing. The rat immunized with Ag and Ag plus adjuvant were almost the same s–IgA concentrations in rats immunized with adjuvants have increased (fig 2). A not much different result also obtained from the ELISA result of serum IgG (fig 3). In contrast to the effects of s–IgA and serum IgG, ELISA result of serum IgA showed an increase in Ag concentration plus adjuvant compared to other treatments (fig 4).



Xb,

Figure 2: ELISA results of sIgA nasal washing Wistar Rats after immunized difference letters shown indicate a significant ($p < 0.05$)

Data from the ELISA results were then analyzed using one way ANOVA. The results of the analysis show that there is a significant difference between immunized and combined antigen–adjuvant compared to the other group in AI and IgA but not the substantial difference in serum IgG.

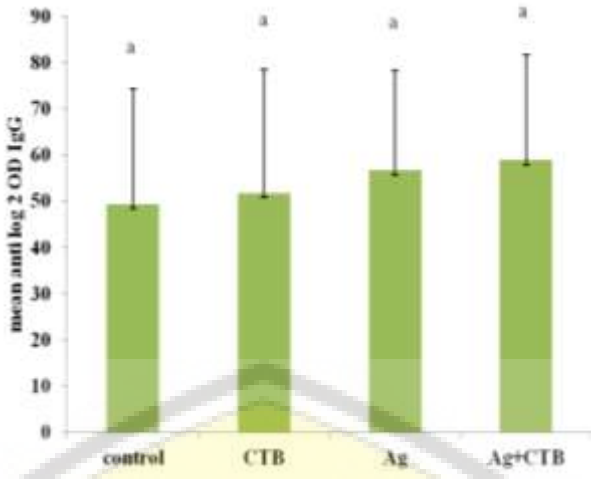


Figure 3: ELISA results of IgG serum Wistar Rats after immunized difference letters shown indicate a significant ($p < 0.05$)

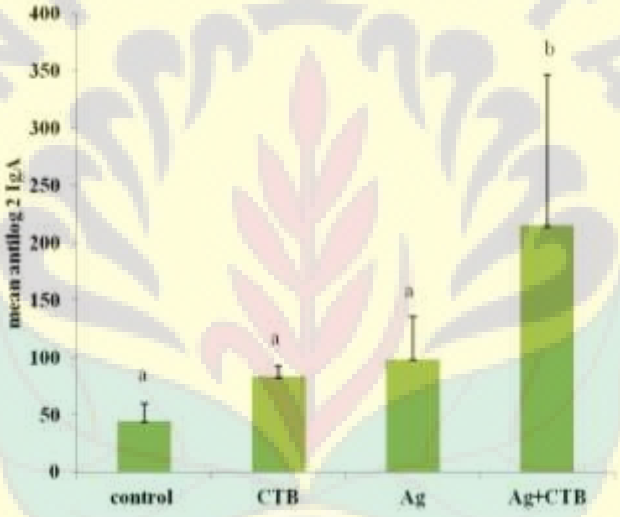


Figure 4: ELISA results of IgA serum Wistar Rats after immunized difference letters shown indicate a significant ($p < 0.05$)

Discussion

The results of the identification of the pili *S. pneumoniae* protein in figure 1 indicate the presence of several proteins with molecular weights including (67, 54, 25, 11) kDa. The four proteins are likely to be immunogenic, according to Parslow [13] statement that proteins with molecular weights between 10 kDa to 100 kDa have immunogenic properties [13]. Protein which has a high antigenicity used in this study is 54 kDa pili protein [4]. This protein is a material that has been developed as a vaccine, especially for mucosal vaccination.The mucosal surface is the main entrance for infectious pathogens, and therefore the mucosal immune response serves as the first line of defense. Most immunization procedures are now obtained by parenteral injection, and only a few vaccines are given via the mucosal route, because of their low efficiency. However, in fact, from several studies showing that mucosal vaccination can induce antigen–specific humoral and cell–mediated immune responses in both the systemic and mucosal compartments. Additionally, such vaccination efficiently induces long–lasting B and T–cell memory. Mucosal vaccination has some physiological and practical advantages, such as reduced costs and reduced risk of needle sticks and transmission of blood diseases, and it is stainless. Importantly, the characteristics of mucosal immune response induction depend on the delivery route, for example, nasal mucosa [14–16]. Nasal mucosa is a suitable place for administering vaccines against respiratory infections not only because the nasal cavity is the entry point for respiratory pathogens, but also because of the immune system mucosa of the respiratory tract by the

NALT. NALT is key organized lymphoid structures in the respiratory tract and ocular cavities, respectively, and has been shown to interact with each other. Intranasal vaccines such as FluMist, a live attenuated influenza virus vaccine, generate SIgA in the upper and lower respiratory, gastric, and genital tracts protection against pathogen infection [5–7, 17].

Conclusions

The conclusion of the research shows intranasal immunization 54 kDa hemagglutinin pili proteins of *S. pneumoniae* enhances the concentration of nasal washing sIgA, serum IgA and IgG.

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