Increased levels of IL-10 in the spleen after induction of pili protein 65.5 kDa Klebsiella pneumoniae

Dini Agustina1, Diana Chusna Mufida1, Enny Suswati1, M. Ali Shodikin1, Bagus Hermansyah2, Ajeng Samrotu Sa’adah2, Tio Wisnu Pradana Putra2, Samudra Ayu2

1 Department of Microbiology, Faculty of Medicine, Universitas Jember, Jember, Indonesia
2 Faculty of Medicine, Universitas Jember, Jember, Indonesia

Abstract

Background: One of the gram-negative bacteria often found as the cause of nosocomial infections is Klebsiella pneumoniae. This bacterium has a mortality rate of 28.3% due to ESBL strains that cause resistance to several antibiotics and the absence of a vaccine as a preventive measure. Objective: To determine the level of IL-10 in the spleen after induction of the protein pili K. pneumoniae 65.5 kDa in mice. Method: This study used mice spleen samples from 21 mice aged six to eight weeks in an experimental investigation with a randomised posttest-only control group design. There are three groups, K1 as a PBS-controlled, 65.5 kDa antigen protein pili + Freund’s adjuvants are given to K2, and Freund’s adjuvants are given to K3. Interleukin-10 concentrations were measured using the ELISA and analysed by one-way ANOVA. Result: The research showed significant differences in each group at IL-10 levels after administration of the pili protein K. pneumoniae using one-way ANOVA (p = 0.036). The treatment group had the highest average levels of IL-10, so there was an increased association between exposure to pili protein and IL-10 levels in rats. A significant increase in IL-10 levels was found in the treatment group compared to the control and adjuvant groups. Conclusion: Induction of protein pili Klebsiella pneumoniae 65.5 kDa increases IL-10 levels in spleen mice.

Introduction

Klebsiella pneumoniae is a rod-shaped gram-negative bacterium that is opportunistically pathogenic. This bacterium is the leading cause of healthcare-related (nosocomial) infections, primarily affecting individuals with low immune systems or suffering from concurrent bacterial infections. However, with the dramatic increase in hyper-virulence strains and the emergence of new strains resistant to antibiotics, the mortality rate and incidence of infections due to these bacteria are increasing, especially in low- and middle-income countries, one of the cases whose main cause of this bacterium is neonatal sepsis. These microorganisms account for neonatal sepsis in 4-9% of cases in developed countries and 16-28% in developing countries. In addition to hypervirulence strains, in K. pneumoniae, there is also a “classic” strain (classic K. pneumoniae/c-KP). This non-virulent strain is usually associated with pneumonia, urinary tract infections, nosocomial infections, and neonatal sepsis in immunocompromised patients. C-KP can also cause neonatal sepsis outbreaks in hospitals and ICUs (Camacho-Gonzalez et al., 2013; Shon et al., 2013; Haller et al., 2015; Zea-Vera & Ochoa, 2015; Khartynov et al., 2016; Verma et al., 2017; Khartynov et al., 2018; Arato et al., 2021).

A risk factor for neonatal sepsis is ineffective therapy in nosocomial infections due to antibiotic resistance (Al-Hasan et al., 2011; Weiner, 2016). Antibiotic resistance of the K. pneumoniae strain is associated primarily with the Enzyme Extended Spectrum β-Lactamase (ESBL) production. In recent decades,
isolates of K. pneumoniae ESBL have been found worldwide, especially in intensive care units (ICUs). The prevalence of ESBL-producing strains of K. pneumoniae is 23% in the US and up to 85-100% in some European countries (The state of the World’s Antibiotics, 2015 - Center for Disease Dynamics, Economics & Policy (CDDEP), n.d.). In 2017 the World Health Organisation included ESBL-producing K. pneumoniae in its list of the most dangerous superbugs along with acinetobacter baumannii and Pseudomonas aeruginosa (WHO Releases List of World’s Most dangerous superbugs - scientific American, n.d.). The dramatic increase in microbial resistance to antimicrobial agents and the lack in new antimicrobials have limited therapeutic options against these pathogens, further exacerbating the disease burden and resulting in an increase in morbidity and mortality (Assoni et al., 2021; Choi et al., 2020). The right solution to solve this problem is a vaccine.

Vaccines can reduce the incidence of infection, reducing the need for antimicrobials and not being affected by antimicrobial resistance mechanisms. No vaccine has been licensed by the Food and Drug Administration (FDA) (Choi et al., 2020; Lundberg et al., 2013a). Although there is a lot of evidence that supports the protection of antibodies against polysaccharide capsules, the large number of types causes vaccines from this virulence factor to be less feasible, so it is necessary to develop vaccines from other virulence factors such as fimbriae/pili (Arato et al., 2021; Choi et al., 2020; Lundberg et al., 2012). Our previous research showed that the pili protein K. pneumoniae 65.5 kDa exhibits immunogenic properties, which can hypatinate erythrocytes and can act as adhesin (Agustina et al., 2014, 2020; Kurniawati et al., 2021; Thanassi et al., 2012), as well as being able to induce cytokines as a sign of the emergence of an immune response (in editing). The cytokine used in this study was interleukin-10 (IL-10). Interleukin-10 is an anti-inflammatory cytokine produced during a bacterial infection, acting as an immunomodulator that affects the innate and adaptive immune systems. These cytokines are necessary for bacterial clearance, reduction of tissue damage, and survival of hosts (; Duell et al., 2012; Ng et al., 2013; Peñaloza et al., 2018; Decker et al., 2019).]

In addition to IL-10, this study also used the spleen; in contrast to previous studies, the examination of serum shows that pili protein 65.5 kDa K. pneumoniae decreases the IL-10 level (Agustina et al., 2022). IL-10 functions as a mediator of immune responses, with NK (Natural Killer) cell intermediaries as the leading primary producers of IL-10. This immune reaction can be measured by looking at increased levels of Interleukin-10 with an Enzyme-linked immunosorbent assay (ELISA). Based on the above statement, researchers want to determine the levels of IL-10 in the spleen after the induction of the pili protein K. pneumoniae 65.5 kDa in mice.

**Methods**

**Design**

This study is purely experimental, with a post-control-only group design and random sampling techniques. In this study, 21 mice spleen samples aged 6-8 weeks with a body weight of approximately 25 grams were used, which were divided into three groups. The first group was the control group with Phosphate Buffer Saline (PBS) administration, the second group was the treatment group with pili protein administration, and the third group was the adjuvant group with the administration of CFA (Complete Freund’s Adjuvant) andIFA (Incomplete Freund’s Adjuvant). The K. pneumoniae bacteria used are the culture stocks of the microbiology laboratory, Faculty of Medicine, University of Jember.

This study’s working procedure consisted of identifying the K. pneumoniae 65.5 kDa pili protein using SDS-PAGE electrophoresis based on the Laemmli method (Blancher & Jones, 2003; Brunelle & Green, 2014; Laemmli, 1970; SCHÄGER, 2003). After identification of the desired protein band, propagation and purification are then carried out and measured using the Kingsley method (Kingsley, 1939; Sukarjati et al., 2018). The purified protein was induced intraperitoneally in group 2 mice three times with an interval of 14 days. The adjuvant that was first given was CFA, then as a booster, was given IFA. Mice are terminated and taken spleen after 14 days of administration of the second booster (Care & Committee, 2017; Greenfield, 2020, 2019). The prepared spleen is washed, crushed, and homogenised with PBS; the last step is centrifugation in 2000-3000RPM for 20 minutes.

**Assessment**

IL-10 measurements were carried out by the ELISA method with the Bioassay Technology Laboratory kit. The measurement results are then analysed using one-way ANOVA (CUSABIO, n.d.; Setiawan & Nugraha, 2016). The steps in the study can be seen in Figure 1.
Results

In the Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) gel, the thickest protein. The band is a protein band with a molecular weight of 65.5 kDa (see Figure 2), while the results of measuring the purified protein reach 0.65 g/dl. After the induction procedure and preparation of the spleen sample, standardisation is conducted first before measurements with ELISA, with the results seen in Figure 3.
The results of measuring IL-10 levels using ELISA can be seen in Figure 4. The figure showed an increase in IL-10 levels in the treatment group given 65.5 kDa pili protein _K. pneumoniae_, with mean and standard deviation for each group shown in Table I. In addition, there were significant differences in each group at IL-10 levels after administration of the pili protein _K. pneumoniae_ using one-way ANOVA ($p = 0.036$). The treatment group had the highest mean IL-10 levels, so there was an increased relationship between exposure to pili protein and IL-10 levels in mice. A significant increase in IL-10 levels was found in the treatment group compared to the control group. It was also found in the treatment group compared to the adjuvant group (see Table II).

**Discussion**

_Klebsiella pneumoniae_ infection, which causes an increase in morbidity and mortality so that it becomes a heavy burden on the economy, especially in developing countries, causes the development of vaccines to prevent this infection to be necessary. The development of the _Klebsiella_ vaccine has focused on five virulence factors: capsules, lipopolysaccharide (LPS), siderophores, adhesions, and exotoxin. Among the five types of vaccines that protein-based vaccines focus on, immunogenic proteins are outer membrane proteins and fimbriae/pili proteins (Lundberg et al., 2013b). The pili protein _K. pneumoniae_ generated a host immune response shown by an increase in mean...
levels of IL-10 in the treatment group, which exceeded the mean value of the control group. This can happen because the pili protein triggers T cells and B cells to opsonize the pili protein and causes an increase in levels of IFN (Interferon) as a pro-inflammatory. Humoral responses (antibodies) are important in controlling K. pneumoniae infection. In mice that have been infected with K. pneumoniae, anti-K antibodies pneumoniae mice cause a protective response in the form of helper T cells that eventually become IL-10 (Pennini et al., 2017). Infection is considered nonexistent if there is no increase in IL-10 levels, and the administration of the pili protein K. pneumoniae as a trigger for the formation of the immune system is considered to have failed (Sudiono, 2014).

The Post Hoc test showed significant differences in the treatment group compared to the adjuvant group. This result is because the immunogenic levels of pili proteins are much higher than adjuvants. Pili protein triggers the immune system specifically by forming an anti-K. pneumoniae. Adjuvants using Freund’s adjuvant trigger the immune system thoroughly and nonspecifically. This makes the difference in mean IL-10 levels between the treatment and adjuvant groups. There is a significant difference (Gregoriadis et al., 2018).

Another study by Won-Hee Lee (2015) obtained a significant increase in IL-4 levels in a family with IL-10. IL-4 functions as an anti-inflammatory, producing memory B cells that will be circulated throughout the body. Won-Hee Lee’s study used extracellular vesicles from K. pneumoniae injected intra-peritoneally into mice to find the best vaccine candidate. However, in this study, the mortality rate from mice was still too high with the administration of a lethal dose of K. pneumoniae (1x10^8 CFU), so further research is still needed (Lee et al., 2015). A similar study by Gaowei Hu (2022) compared the effectiveness of the administration of K. pneumoniae by IWB (inactivated whole bacteria) with the outer membrane phosphoporin with 3x intraperitoneal administration. This study used a strain with a molecular weight of 38 kDa and obtained an increase in IL-4 3x higher than normal, and immunised mice were given K. pneumoniae at a lethal dose. IL-4 is still a family with IL-10 with almost the same effect. Mice immunised with IWB and outer membrane phosphoporin have a high survival rate with minimal damage to mice. This research proves outer membrane protein be used as a vaccine candidate (Hu et al., 2022).

Previous studies on mice serum proved that 65.5 kDa K. pneumoniae protein pills that lowered IL-10 levels when administered together with the Freund adjuvant did not prove to be the expected immunogens compared to the control group (Agustina et al., 2022). So, the results of this study can contribute to efforts to make protein-based vaccines against K. pneumoniae, especially in proving the ability of these proteins to induce cytokines, especially IL-10.

The limitation of this study was not differentiating doses between and within the group and not conducting a microscopic assessment to see whether there was damage and specific protein expression in spleen mice infected by Klebsiella pneumoniae.

Conclusion

Induction of the protein pili Klebsiella pneumoniae 65.5 kDa intraperitoneally increases the levels of IL-10 spleen mice.

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