Interferon-Gamma profile of mice (\textit{Mus musculus} L.) after complete SARS-CoV-2 vaccination

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Abstract---Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the resulting coronavirus disease 2019 (COVID-19) have afflicted millions of people in a worldwide pandemic. Several vaccines have been developed to prevent infection and illness. The success of preventing and controlling the COVID-19 by using an effective and efficient vaccine. This study aimed to find out the profile of interferon-gamma (IFN-γ) after COVID-19 vaccination. This study conducted at Animal Laboratory Professor Nidom Foundation (ABSL-3) from December 2021 to February 2022. The present study is an experimental study with a post test only control group design using 90 mice (Mus musculus L.). The test group consisted of mice injected subcutan with 0.1 mL PBS (P1), 0.1 mL adjuvant 1 (P2), 0.1 mL adjuvant 2 (P3), 0.1 mL VLPs SARS-CoV-2 10 μg (P4), 0.1 mL VLPs SARS-CoV-2 dose 50 μg (P5), 0.1 mL VLPs SARS-CoV-2 10 μg + adjuvant 1 (P6), 0.1 mL VLPs SARS-CoV-2 10 μg + adjuvant 2 (P7), 0.1 mL VLPs SARS-CoV-2 50 μg + adjuvant 1 (P8), and 0.1 mL VLPs SARS-CoV-2 50 μg + adjuvant 2 (P9). The data analysed by using ANOVA. The result showed that there was a significant difference between all test group and based on the results of the specific humoral immune response test, the effect of the COVID-19 vaccine was found in the form of an increase in IgG and IFN-γ profile from group 2 to group 9. In summary, this study successfully concluded that formulation COVID-19 vaccine can proceed to next step.

Keywords---COVID-19, virus-like particles, adjuvant, IFN-γ, IgG.

Introduction

The World Health Organization (WHO) has declared the emergency caused by SARS-CoV-2 as a pandemic outbreak on March 11, 2020 (Nidom et al., 2020a; Nidom et al., 2020b; Nidom et al., 2021). WHO’s consideration was due to the rapid progression of the disease and the increasing number of exposed patients (Ansori et al., 2020; Ansori et al., 2021; Nur et al., 2022). As of May 2020, the coronavirus has infected approximately 28 million people worldwide with more than 900,000 deaths worldwide. In addition, there are more than 200,000 cases and about 8,500 deaths in Indonesia. This data is based on the Johns Hopkins University online website that tracks COVID-19 cases in real-time (Dong et al., 2020).
SARS-CoV-2 infection in severe cases causes tissue damage and will activate and stimulate the immune response. Infection by the virus activates an exaggerated immune system response including macrophages, granulocytes as well as proinflammatory cytokine production, CD4+ T, and CD8+ T cell activation with the aim of controlling viral replication, limiting viral spread, inflammation, and clearance. However, excessive immune response will cause tissue damage and inflammation known as cytokine storm. If proinflammatory cytokines continue to be produced, it will worsen the course of the disease and prognosis and death in patients (Kordzadeh-Kermani et al, 2020).

The successful prevention and control of COVID-19, one of which is by using an effective and efficient vaccine. Referring to the regulations and standardization from WHO in 2003 that vaccines are included as a type of drug, so vaccines must pass immunogenicity tests. The immunogenicity test starts from pre-clinical testing and continues with clinical testing, after the pre-clinical test is decided to meet the standards. Laboratory testing of the immune response that occurs is one of the benchmarks of pre-clinical testing. Immunogenicity test parameters include humoral immune cell testing (antibody titer), cellular (cytokines), and antibody protectiveness against natural viruses (PRNT). Testing the cellular immune response, one of which includes examining the IFN-γ profile, measuring the IFN-γ profile in the body can provide important information about the immune response mechanism of vaccination (Ashmawy et al., 2022; Banerjee et al., 2022; Hernández-Bernal et al., 2022; Vajo, 2022; Xu et al., 2021).

Enzyme-linked immunospot (ELISpot) assay is an immunoassay with very high sensitivity (Axelsson, 2022; Lin et al., 2022; Tan et al., 2022). So far, there has been no research on SARS-CoV-2 vaccine formulation in mice using the ELISpot method to count each cytokine-secreting cells. Cytokine testing using the ELISpot method can be used to determine vaccine effectiveness by measuring the capacity to elicit a strong immune response. The information obtained can then be used to evaluate appropriate planning for vaccine design.

**Methods**

**Experimental animal and research design**

The research protocol was approved by the Institutional Animal Care and Use Committee of the Professor Nidom Foundation (IACUC-PNF) (approval number: 011121/IACUC/VII/2021; 20 November 2021). The research design used was laboratory experimental, using experimental animals as the object of research. The animals used were 90 female mice (*Mus musculus* L.) aged 6-8 weeks with a body weight of 20-30 grams which were divided into nine groups randomly and each group consisted of ten animals. The study was designed as follows: 0.1 mL PBS (P1), 0.1 mL adjuvant 1 (P2), 0.1 mL adjuvant 2 (P3), 0.1 mL VLPs SARS-CoV-2 10 μg (P4), 0.1 mL VLPs SARS-CoV-2 dose 50 μg (P5), 0.1 mL VLPs SARS-CoV-2 10 μg + adjuvant 1 (P6), 0.1 mL VLPs SARS-CoV-2 10 μg + adjuvant 2 (P7), 0.1 mL VLPs SARS-CoV-2 50 μg + adjuvant 1 (P8), and 0.1 mL VLPs SARS-CoV-2 50 μg + adjuvant 2 (P9). All injected subcutaneously with a volume of 0.1 mL on day 0 and day 21 (booster).
Technique of collecting the data

After 35 days of treatment, all animals were euthanized using a mixture of ketamine and xylazine administered intramuscularly. After that, the animals were euthanized, mixture of ketamin and xylazine. Then, the intracardial blood was taken with a disposable syringe. The blood was then put into EDTA vacutainer tubes, PBMC preparation, and ELISpot examination.

Enzyme-linked immunospot (ELISpot) assay

For the detection of drug-specific IFN-γ producing T-lymphocytes, a pre-coated a mouse IFN-γ ELISpot plus kit (HRP) batch 50 (Mabtech Code 3321-4HPT-2, USA) was used. ELISpot examination begins with preparing materials and ELISpot plates in a sterile state, opening the seal on a new plate and washing the plate with 200 μL/well sterile PBS. Then fill the plate with 200 μL/well media containing 10% serum and incubate 30 minutes at room temperature. The next stage is incubation of cells on a plate (sterile conditions) by replacing the previous media and adding stimul and cell suspensions then placed in a humidified incubator 37 °C with 5% CO₂ for 12-48 hours. The last step is spot detection, by emptying the plate washing five times with PBS 200 μL/well. Dilution of detection antibody (R4-612-biotin) 1 µg/mL into PBS containing 0.5% fetal calf serum (PBS-0.5% FCS). Next, we added the solution as much as 100 μL/well and incubate for 2 hours at room temperature. Then, washed the plate and dilute streptavidin-ALP (1:1000) to PBS 0.5% FCS and add 100 μL to each well and incubate for 1 hour at room temperature. Next, adding 100 μL/well of ready-to-use substrate solution (TMB substrate solution) until distinct spots appear. Then, stop the color development by washing extensively with distilled water and then drying the plate. Finally, check and count on the ELISpot reader. Results were expressed as a) the number of IFN-γ SFC/2.5×10⁶ PBMC for the unstimulated condition (medium); b) values, the difference between the response value in the presence of stimulated by LPS and the unstimulated condition value.

Statistical analysis

Data analysis using multivariate ANOVA with IBM SPSS Statistics version 26 (IBM Inc., USA), p value less than 0.05 was considered significant.

Results and Discussion

In this study, we revealed that the highest mean IFN-γ profile was obtained in the treatment group injected with 50 µg VLPs (P5) and the lowest in the treatment group injected with PBS (P1). The mean IFN-γ profile of mice between treatments P1, P2, P6, P3, and P8 had no significant difference (p<0.05). IFN-γ profile of mice between treatments P1, P6 with treatment P4, P7 there is a significant difference (p<0.05), while between P8 and P5 there is no significant difference (p<0.05). Table 1 showed that there are differences in the mean IFN-γ profile of mice (Mus musculus L.) after complete vaccination.
Table 1. Mean IFN Gamma Profile of Treatment Groups 1-9 Mice (*Mus musculus* L.) After Complete SARS-CoV-2 Vaccination

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Mean (SFU/2.5x10^5 sel PBMC) ± Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>PBS (P1) 6.8^a ± 1.48</td>
</tr>
<tr>
<td>VLPs 10 μg</td>
<td>VLPs 50 μg (P4) 51.5^b ± 32.05</td>
</tr>
<tr>
<td>VLPs 50 μg</td>
<td>VLPs 50 μg (P5) 64.8^c ± 78.32</td>
</tr>
<tr>
<td>Adjuvant 1 PBS</td>
<td>PBS (P2) 20.6^ab ± 20.35</td>
</tr>
<tr>
<td>VLPs 10 μg</td>
<td>VLPs 10 μg (P6) 7.7^a ± 3.62</td>
</tr>
<tr>
<td>VLPs 50 μg</td>
<td>VLPs 50 μg (P7) 44.9^b ± 44.86</td>
</tr>
<tr>
<td>Adjuvant 2 PBS</td>
<td>PBS (P3) 15.2^ab ± 11.75</td>
</tr>
<tr>
<td>VLPs 10 μg</td>
<td>VLPs 10 μg (P8) 29.6^abc ± 36.19</td>
</tr>
<tr>
<td>VLPs 50 μg</td>
<td>VLPs 50 μg (P9) 49.6^b ± 39.91</td>
</tr>
</tbody>
</table>

Description: Different superscripts in the same column show significant difference (p<0.05).

Figure 1. Graph of the average IFN-γ profile of mice (*Mus musculus* L.)

Figure 1. After Complete COVID-19 Vaccination. Description: P1: Injected with PBS; P2: Injected with adjuvant 1; P3: Injected with adjuvant 2; P4: Injected with virus like particle SARS-CoV-2 dosis 10 μg; P5: Injected with virus like particle SARS-CoV-2 dosis 50 μg; P6: Injected with virus like particle SARS-CoV-2 dosis 10 μg + adjuvant 1; P7: Injected with virus like particle SARS-CoV-2 dosis 10 μg + adjuvant 2; P8: Injected with virus like particle SARS-CoV-2 dosis 50 μg + adjuvant 1; and P9: Injected with virus like particle SARS-CoV-2 dosis 50 μg + adjuvant 2.
VLPs are non-infectious protein capsids conjugated with viral structural proteins engineered for use in nanotechnology (Cimica & Galarza, 2017). The structure of VLPs corresponds to a natural virus without a viral genome. VLPs are biologically active, strongly immunogenic and adequate to generate humoral and cellular immune systems (Lee & Ashkar, 2018). SARS-CoV-2 VLPs in this study used doses of 10 μg and 50 μg.

However, the immunogenicity test of the COVID-19 vaccine formulation in this study was shown to increase the cellular immune response (IFN-γ). In line with research conducted by Tian et al. (2021) to test the immunogenicity of the SARS-CoV-2 vaccine (NVX-CoV2373) from spike protein in mice, it was found that COVID-19 vaccination with a dose of 10 μg without adjuvant can induce IgG profiles and increase IFN-γ production up to 20× compared to the control group (analyzed using ELISpot).

Based on the IFN-γ profile in mice (Mus musculus L.) after COVID-19 vaccination, the highest mean was obtained in the VLPs 50 μg treatment but after statistical analysis there was no significant difference with the injection treatment of VLPs 10 μg + adjuvant 2 (P7), VLPs 50 μg + adjuvant 1 (P6), VLPs 50 μg + adjuvant 2 (P9), and VLPs 10 μg (P4). This shows that VLPs 10 μg, VLPs 50 μg and adjuvant 2 as additional immunomodulators are able to induce the formation of IFN-γ profiles. IFN-γ itself plays an important role in intracellular defense that will induce macrophage activation and Th1 responses and become a promoter of the overall immune response so it is often referred to as an immunoregulatory distinct cytokine (Lee & Ashkar, 2018).

One of the important roles of IFNγ is in the maturation process of B cells that trigger the formation of antibodies, one of which is IgG (Gao et al., 2008). High IFNγ and IgG production is usually associated with effective host defense against intracellular pathogens. In addition, Moss (2022) stated that on the immune system after COVID-19 vaccination, it was found that antibodies in the body decreased after eight months, although levels varied greatly between individuals, but the number of T cells only decreased slightly and the number of B cells remained stable and sometimes increased even though it was difficult to measure.
This suggests that despite the decline, the components that can restart antibody production and coordinate the attack on the coronavirus persist at a fairly high level, the same mechanisms that lead to the memory of the immune response after infection also form the foundation for immunity after vaccination.

**Conclusion**

In summary, high IFN-γ levels are due to the SARS-CoV-2 formulation that can work to induce the Th1 immune system and NK cells optimally by producing IFN-γ. IFN-γ is a promoter of all immune system regulations when there are viral antigens that enter the body. IFN-γ not only plays a role in innate immunity but also plays a role in adaptive immunity, in the innate immune system IFN-γ produced by NK cells will attract and activate macrophages to produce TNF-α and IL-2 so that more IFN-γ will be produced. IFN-γ also plays a role in the maturation process of B cells that trigger the formation of antibodies, one of which is IgG.

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**References**


