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Protection and profile of immune response against SARS-CoV-2 among the COVID-19 vaccinated and unvaccinated individuals

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Abstract—Coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first reported in the city of Wuhan, China at the end of December 2019. In the case of SARS-CoV-2, antibody-mediated immunity and T cells are the most effective protection. This study aimed to analyze IFN- γ profile in people who were vaccinated and unvaccinated against COVID-19. This research was conducted at the Molecular Laboratory of the Professor Nidom Foundation (LM-PNF), Surabaya, Indonesia from February 2021 to March 2022 using 100 blood samples with details of 50 samples from people who had been vaccinated against COVID-19 and 50 samples from people who had unvaccinated against COVID-19. We divided into four: vaccination only, vaccination and had infected of COVID-19 or survivors, unvaccination only, and unvaccination but survivors. Furthermore, we used the ELISpot method to see the IFN- γ profile. The data analysed by using ANOVA. The results of this study showed that IFN- γ profile vary widely with the highest IFN- γ obtained in samples of people who are vaccinated and had infected of COVID-19 compared to other groups. In summary, we conclude that the cellular immune response (IFN- γ) profile in people who vaccinated and had infected of COVID-19 was better than unvaccinated.

Keywords—COVID-19, ELISpot, IFN- γ , SARS-CoV-2, Vaccination

Introduction

Coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first reported in the city of Wuhan, China at the end of December 2019 (Nidom et al., 2020a; Nidom et al., 2020b; Nidom et al., 2021). Within one month the disease had spread throughout the world. other provinces in China, Thailand, Japan, and South Korea. At first this disease was referred to as 2019-novel coronavirus (2019-nCoV), then on February 11, 2020 World Health Organization announced a new name, namely COVID-19 (Afiahayati et al., 2022; Ansori et al., 2020; Ansori et al., 2021; Nur et al., 2022).

The SARS-CoV-2 vaccination is one of the efforts to reduce morbidity and mortality due to COVID-19 (Nidom et al., 2020a; Nidom et al., 2020b; Nidom et al., 2021). Several vaccine platforms recommended by WHO and some used in Indonesia include inactivated vaccines (Sinovac, Sinopharm), viral vector

(AstraZeneca, Janssen), RNA (Moderna and Pfizer), subunit (Novavax), and dendritic vaccines (Vaksin Nusantara) (Nidom et al., 2020a; Nidom et al., 2021). Any stimulation of the immune response to a vaccine begins with the body's reaction to the first detection of an incoming agent recognized as a threat or immunization (Fathizadeh et al., 2021; Soy et al., 2020). Next the innate immune system performs the initiation stage. The process of initiation and detection begins when the immune system recognizes the epitope of the antigen. In the case of SARS-CoV-2, antibody-mediated immunity and T cells are the most effective protection (Fathizadeh et al., 2021; Soy et al., 2020; Suryasa et al., 2021).

Enzyme-linked immunosorbent spot (ELISpot) is one of the most popular methods used to measure antigen-specific T cells in humans (Wang et al., 2021). ELISpot is a highly quantitative method and can measure various response magnitudes and is able to assess important cellular immune-related activities such as IFN- γ secretion. ELISpot is not only for evaluation of various T cell functions but also for B cells and innate immune cells (Afifah et al., 2022; Tan et al., 2020). Therefore, IFN- γ is called type II interferon which is produced by Th1 cells and NK cells. IFN- γ is the main activator of macrophages, this activation activates macrophages to fight invasive intracellular pathogens (Gauthier & Chen, 2022; Simpson et al., 2022).

Methods

This research according to the data collection is an observational study and according to the data analysis is a descriptive study. In this study, data were taken from testing blood samples using the ELISpot method to unlock the IFN- γ profile. The study was carried out from February 2021 to March 2022. The IFN- γ test using the ELISpot method was carried out at Molecular Laboratory of Professor Nidom Foundation (LM-PNF), Surabaya, Indonesia.

Technique of collecting the data

The blood samples contained in the EDTA/heparin vacutainer tube were obtained from the Molecular Laboratory of the Professor Nidom Foundation, Surabaya, Indonesia by means of total sampling. Furthermore, peripheral blood mononuclear cells preparation and ELISpot test were carried out.

10

Peripheral blood mononuclear cells preparation

Put the whole blood in the EDTA/heparin vacutainer, then the plasma is separated from the red blood cells by centrifuging 1500-3000 rpm for 5-15 minutes, taking the plasma for storage, the red blood cells are used for the next step. The next step is 1:3 blood dilution with 1 \times PBS, carefully put the blood dilution solution into the Ficoll-Paque (3 mL Ficoll: 4 mL blood dilution solution) in a 15 mL conical tube, centrifuge 400 g/500 rpm 30 minutes RT. Take the supernatant in the middle layer between PBS clear liquid and red blood cells, then transfer the supernatant to a new tube. After the supernatant was put into a new tube, add PBS 1 \times 1:1 and centrifuge 250 g/300 rpm 10 minutes RT, discard the supernatant, then dissolve the pellet in the culture medium that had been

given 1 mL of the preservative medium. Finally, stored it at $-80\text{ }^{\circ}\text{C}$ and long-term storage in nitrogen tanks.

Enzyme-linked immunospot (ELISpot) assay

The ELISpot assay begins with preparing the materials and the ELISpot plate in a sterile condition, opening the seal on the new plate and washing the plate with sterile PBS 200 μL /well. Then fill the plate with 200 μL /well medium containing 10% serum and incubate for 30 minutes at room temperature. The next step is incubation of diplate cells (sterile conditions) by replacing the previous medium and adding stimuli and cell suspensions are then placed in a humidified incubator at $37\text{ }^{\circ}\text{C}$ with 5% CO_2 for 12-48 hours (wrap plate with aluminum foil) to avoid evaporation. The last stage is spot detection, by emptying the washing plate 5 times with PBS 200 μL /well. Dilution of detection antibody (R4-612-biotin) 1 $\mu\text{g}/\text{mL}$ onto PBS containing 0.5% fetal calf serum (PBS-0.5% FCS). Next, add the solution as much as 100 μL /well and incubate for 2 hours at room temperature. Then, wash the plate and dilute streptavidin-ALP (1:1000) to PBS 0.5% FCS and add 100 μL to each well and then incubate for 1 hour at room temperature. Next, filter the ready-made substrate solution (ALP/BCIP) through a 0.45 m filter and add 100 μL /well until distinct spots appear. Then, stop color development by washing extensively with tap water and then drying the plate. The last is to check and calculate in the ELISpot reader (Afifah et al., 2022).

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 (Afifah et al., 2022).

Results and Discussion

The sample of this study was obtained as many as 100 samples with details of 50 samples from people who had been vaccinated against COVID-19 and 50 samples from people who had unvaccinated against COVID-19. Based on data from the client sample information sheet of Molecular Laboratory of Professor Nidom Foundation, 10 samples have been vaccinated against COVID-19 and have been infected with the SARS-CoV-2 as evidenced by positive results on RT-PCR, 14 samples have unvaccinated but had been infected with the SARS-CoV-2.

Table 1. Distribution of Vaccinated and Unvaccinated Samples for COVID-19

No	Historical of Vaccinated	Sampel Size	Gender		History of COVID-19 Infection	
			Male	Female	Infected	Uninfected
1	Vaccinated Against COVID-19	50	24	26	10	40
2	Unvaccinated	50	24	26	14	36
	Quantity	100	48	52	24	76

Regarding the type of vaccination used in the sample of people who vaccinated against COVID-19 showed that 18 samples (36%) received inactivated vaccines (Sinovac, Sinopharm, etc.), 8 samples (16%) received mRNA vaccines (Pfizer, Moderna, etc.), 10 samples (20%) received non-replicating viral vector vaccines (Astrazanece, etc.), 11 samples (22%) received dendritic-based vaccines (Vaksin Nusantara), and 3 samples (6%) did not mention the type of vaccination used or unknown.

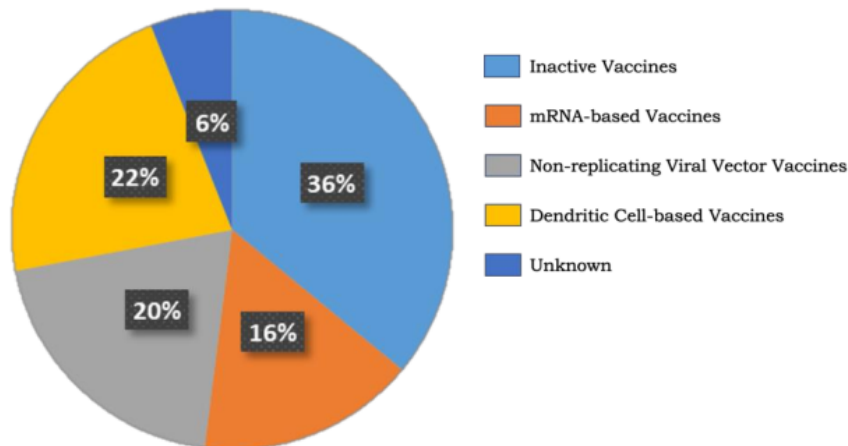


Figure 1. Graph data of vaccine type had received by vaccinated against COVID-19 samples

In this study, we divided into four namely vaccination only, vaccination and survivors, unvaccination only, and unvaccination but survivor. We did not differentiate between age, sex, or the type of vaccine used. In addition, the results of the IFN- γ profile were obtained from the ELISpot test using lipopolysaccharide (LPS) [Cat No. 124-80-10MG] (Sigma Aldrich, USA) as a stimulant showed varying results both in people who had been vaccinated against COVID-19 and in people who had not vaccinated against COVID-19. From 100 samples tested, the sample of people who vaccinated against COVID-19 obtained IFN- γ results with the lowest value of 0 SFU/ 2.5×10^5 cells and the highest of 9 SFU/ 2.5×10^5 cells, in the sample of people who vaccinated against COVID-19 and survivors obtained IFN- γ results with the lowest value of 0 SFU/ 2.5×10^5 cells and the highest of 9 SFU/ 2.5×10^5 cells, in samples of people unvaccinate against COVID-19 the results of IFN- γ were obtained with the lowest value of 0 SFU/ 2.5×10^5 cells and the highest was 3 SFU/ 2.5×10^5 cells, while for people who unvaccinate against COVID-19 but survivors, the lowest value was 0 SFU/ 2.5×10^5 cells and the highest was 2 SFU/ 2.5×10^5 cells.

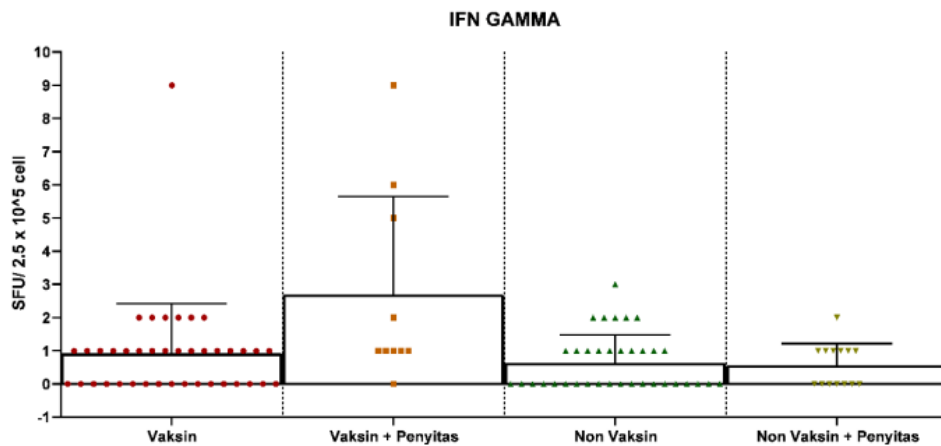


Figure 2. Graph of IFN- γ profile from vaccinated and unvaccinated against COVID-19 samples

The results of data analysis and statistical tests related to the IFN- γ profile showed that there was a significant difference between samples of people who were vaccinated against COVID-19 and samples of people who were vaccinated against COVID-19 and survivors with a P value of 0.001 ($P < 0.05$). In addition, there was also a significant difference between samples of people who were vaccinated against COVID-19 and survivors with samples of people who were not vaccinated against COVID-19 and samples of people who were not vaccinated against COVID-19 and survivors with a P value of 0.000 ($P < 0.05$). The Duncan's post hoc test showed that the sample of people who vaccinated against COVID-19 and survivors was the best on IFN- γ results as shown in Figure 2.

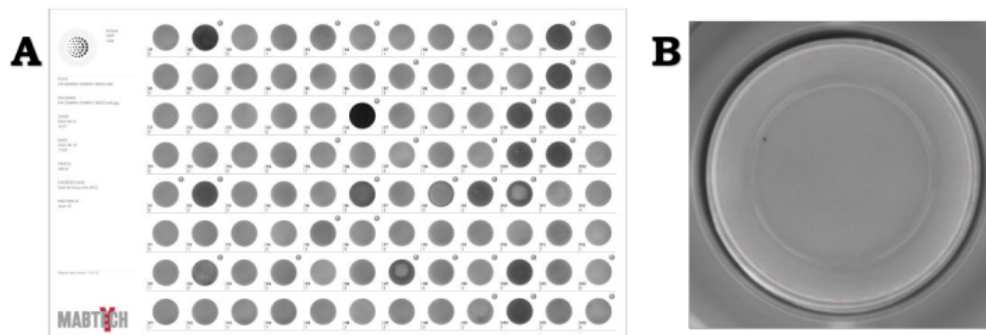


Figure 3. A. Results on the ELISpot reader (all-well); B. Picture of ELISpot Well results sampel cell, found 1 Spot Forming Units (1 SFU/2.5 x 10⁵ cells)

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 (Han et al., 2020; Luo et al., 2020; Zhang et al., 2020). The results of the higher IFN- γ profile were obtained in the sample group of people who were vaccinated and survivors (Congrave-Wilson et al., 2022).

¹⁸ This is in line with the research conducted by Afifah et al. (2022) revealed that the mice given a complete SARS-CoV-2 vaccine formulation, showing that the immunogenicity test of the COVID-19 vaccine formulation in this study was shown to increase the cellular immune response (IFN- γ).

However, ⁹ in cases of severe SARS-CoV-2 infection, the concentrations ¹⁴ of IL-6, IL-10, IL-2 and IFN- γ were higher in serum than in mild cases, indicating that the large number of cytokines was associated with the severity of the disease. In addition, IFN- γ system ¹⁵ is essential for antiviral defense. IFN- γ downregulate virus replication and it activates cytokine production ⁸ T cells, augmenting the cytotoxic T lymphocyte killing activity. Furthermore, persistent high levels of IFN- γ worsens the systemic inflammation, and increasing tissue injury and organ failure (Gadotti et al., 2020).

Conclusion

In summary, the ²¹ interaction between the host and SARS-CoV-2 that causes infection involves a complex response from the immune system. The results showed that samples of people who were vaccinated against COVID-19 and survivors had a better IFN- γ . IFN- γ is a promoter of all immune system ¹⁶ regulations when there are viral antigens that enter the body. IFN- γ not only plays an important role in innate immunity but also in adaptive immunity.

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