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Analysis of cellular immune response in adults after administration of the coronavirus disease 2019 vaccine using the virus vector platform (Astra Zeneca)

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Abstract

Coronavirus Disease 2019 (Covid-19) is caused by a novel virus that has never been identified or detected in humans before. To date, there is no specific treatment available for managing Covid-19 infection. To analyze the cellular immune response in adults 12 months after receiving the Covid-19 vaccine using the viral vector platform (AstraZeneca) as an alternative method to evaluate vaccination success. (AstraZeneca). The research sample consists of a group of recipients of the viral vector platform vaccine (AstraZeneca) for a period of 12 months since the second dose. Blood samples will be taken, followed by PBMC isolation and T cell immunophenotyping to calculate T cell and B cell subpopulations. Relevance of the research topic to the faculty's research roadmap: This study will explore the role of viral vector vaccination in the immune response to Covid-19. This objective is closely related to and supports the achievement of the 2016-2020 Strategic Plan and Master Plan for Research at Trisakti University, with a focus on biomedical and health behavior research. Preliminary results: The subjects involved in this study are mostly women, aged 36-45 years. They have received a third dose (booster) of the vaccine, have not been infected with Covid-19 in the past year, and show indications of prehypertension based on blood pressure measurements at the time of sampling. The patients' blood samples have undergone PBMC isolation and are currently stored. Immunophenotyping will be conducted to determine the T cell and B cell subpopulations using flow cytometry, which is currently in the optimization stage. Preliminary The subjects involved in this study are mostly women aged 36-45 years. They have received a third dose of the vaccine (booster), have not been infected with COVID-19 in the past year, and show indications of prehypertension based on blood pressure measurements taken at the time of sampling. Some of the samples that have undergone cellular immune response analysis show a percentage of CD4+ and CD8+ T cells of around 70%.

Introduction

Coronavirus Disease 2019 (Covid-19) is caused by a new type of virus that has never been found or identified in humans before. This infection was first discovered in the city of Wuhan, China(Yu et al., 2020) . *The World Health Organization (*WHO) has named the source of the infection *Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), while the disease is referred to as Coronavirus Disease 2019 (COVID-19*). The number of Covid-19 cases continues to grow and it has taken only a relatively short time for the disease to spread to almost all countries in various parts of the world(Yu et al., 2020; Chams et al., 2020) . In line with the increasing number of cases in all countries, the WHO officially declared Covid-19 a pandemic on March 11, 2021(Li et al., 2020) . In Indonesia, the first confirmed case of SARS-CoV-2 infection was reported on March 2, 2020, and the number of cases continues to increase to this day(Khariri, 2020) .

Before the emergence of Covid-19, two types of coronaviruses had already been identified as the source of infection in major epidemics over the past two decades. These two viruses are SARS-CoV, which was also first discovered in China around 2002-2003. In addition, there is *the Middle East Respiratory Syndrome Coronavirus (*MERS-CoV), which was discovered in Saudi Arabia in April 2012. These three coronaviruses are suspected to be zoonotic and can cause severe infections and death in humans(Guo et al., 2020; Elrashdy et al., 2020). Coronaviruses have great genetic diversity and frequent genome recombination. Additionally, the unavoidable interaction between humans and animals, particularly in activities related to agriculture and plantation work, leads to the potential emergence of new and evolving Coronavirus strains that become sources of seasonal infections regularly (Guo et al.,

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2020) .

A person can contract Covid-19 infection through close contact with an infected person, contaminated objects or environments, droplets, or *airborne transmission*. When a Covid-19 patient expels droplets, they can travel up to 1-2 meters. *Airborne* transmission can travel even further. Transmission often occurs unnoticed when we are in public places. When we are in a place where the virus is present, it can attach itself to our clothes or the objects we use. These objects can then become a source of transmission for people who live in the same house, even if they do not leave the house. Being in a crowd or in a crowded place also puts you at risk of contracting Covid-19.

Transmission is also possible for those visiting areas categorized as virus circulation zones or pandemic areas(Yuen et al., 2020; Kaur et al., 2020; Zimmermann & Curtis, 2019) . To date, there is no specific treatment available for managing Covid-19 infections. Scientists continue to conduct research and development to find the best ways to mitigate the spread and treat COVID-19 infections. To date, effective therapy for COVID-19 patients is not yet available, and several studies related to this are still ongoing. Every individual and community behavior aimed at preventing transmission is an effort to break the chain of transmission and reduce the number of infections. One of the measures to prevent COVID-19 infection is through vaccination (Andersem et al., 2020).

COVID-19 vaccination is an artificial immune system that is deliberately obtained by exposing antigens in an effort to actively boost a person's immune system against the threat of SARS-CoV-2 infection. A person who has received the COVID-19 vaccine will have antibodies so that if exposed to SARS-CoV-2, they will not develop symptoms or the symptoms that do appear will be milder. Vaccination aims to prevent infection by a microorganism in an individual. Through vaccination, the body is trained to produce antibodies as an immune response to infection by introducing specific substances that can be recognized by the body. Antibodies are formed after vaccination as an immune response to infection by introducing specific substances that can be recognized by the body(Tang et al., 2020) .

Vaccination plays a role in introducing antigens to stimulate the production of specific antibodies that will last for a long time. Someone who has been vaccinated and infected with COVID-19 will have a specific immune system that will quickly form antibodies that can fight the disease. The adaptive immune response formed due to vaccination can be divided into two types, namely cellular and humoral. The humoral immune response is necessary for the host's defense mechanism because it is the first line of defense, so that a person will have antibodies that can resist infection. During embryogenesis, cytotoxic T cells play a role in the cellular immune response. T cells migrate from the bone marrow to the thymus and undergo differentiation into immunocompetent cells (Deng & Peng, 2020).

Vaccination is an effort to reduce the spread, reduce morbidity and mortality due to Covid-19, build *herd* immunity in the population, and protect against the threat of Covid-19 so that community activities can continue. *Herd immunity* is indirect protection from the threat of certain infectious diseases. This occurs when a population has immunity acquired through vaccination or immunity developed from a previous infection. *Herd immunity* reduces the likelihood of individuals becoming infected when in contact with vulnerable individuals. *Herd immunity* can be achieved if vaccination coverage is high (>70%) and widespread across all regions (He et al., 2020; Chan et al., 2020).

Indonesia began its COVID-19 vaccination program in January 2021. Vaccination data as of March 4, 2022 shows that 196,880,116 (94.53%) of Indonesia's population has received the first dose of the COVID-19 vaccine, 160,107,111 (76.88%) have received their second dose, and 24,045,810 (11.55%) have received their thirddose. The vaccination targets have been distributed among 1,468,764 health workers, 21,553,118 elderly people, public officials (17,327,167), vulnerable and general communities (141,211,181), the 12-17 age group (26,705,490), and the 6-11 age group (26,400,300). The COVID-19 vaccination program in Indonesia has been ongoing for 15 months and has been conducted in two phases. Period 1 ran from early January to late April 2021. Vaccinations during period 1 were prioritized for 1.3 million health workers and 17.4 million public officials in 34 provinces. Phase 2 lasted for 11 months,

from April 2021 to March 2022, and began to reach the general public, including the elderly (aged 60 and above), those aged 50 and above, followed by those aged 12 and above, with a vaccination coverage target of 181.5 million people (Wu et al., 2020).

The effectiveness *of herd immunity* can be influenced by several factors, such as vaccine efficacy, the duration of immunity formed, including neutralizing antibodies and memory cells, and the threat of new *variants of concern* (VoC) emerging. The emergence of several variants poses a challenge in vaccination programs. Each Covid-19 vaccine has its own characteristics, such as the number of doses, the interval between doses, and the platform, which also vary. Some of the platforms used in the development of COVID-19 vaccines include *inactivated* or *attenuated vaccines, subunit vaccines,* mRNA *vaccines*, and *viral vector-based* vaccines (Zhou et al., 2020).

Inactivated vaccines express various native viral antigens, including surface antigens with epitope conformations that will induce an antibody response. The manufacture of inactivated vaccines requires additional ingredients and repeated administration to achieve the desired effectiveness. Inactivated vaccines cannot induce CD8 T cells well and will induce a T helper 2 - mediated immune response. Viral vector vaccines are created with replication deficiency or weakened replication through biological engineering to express the target antigen. These vaccines are already widely used, although only a few viral vectors have been approved for use in vaccine production. Most viral vector vaccine candidates express the S protein or RBD of SARS-CoV-2. These vaccines generally induce a strong immunological response and do not produce infectious particles, making them safe to administer(Zhou et al., 2020) .

Viral vector vaccines are endocytosed by APC cells. Immune stimulation by viral vector immunogens can induce the NOD-like receptor family pyrin domain-containing (NLRP) 3 pathway, inflammasome activation, and cytokine production. Transgenic vector-encoded genes are transcribed to produce immunogenic proteins, which are then processed by proteasomes and associated with MHC-I or MHC-II in endocytic vesicles. MHC-I molecules loaded with transgenic epitopes translocate to the cell membrane and are recognized by antigen-specific CD8+ T cells. This results in the killing of infected cells and the release of antigens into the extracellular space. Similarly, MHC-II molecules loaded with transgenic epitopes and translocated to the cell membrane are recognized by CD4+ helper T cells that secrete cytokines and chemokines and subsequently activate antigen-specific CD8+ T cells and B cells. Stimulated B cells mature into plasma cells that secrete antibodies and/or memory B cells, and some stimulated T cells become memory cells. Overall, live immunogens are capable of stimulating both humoral and cellular immune responses (Jiang et al., 2020) .

In whole virus vaccine-induced immune stimulation, the antigen inoculated together with the adjuvant will induce cytokine production from local cells. Cytokines will activate or attract APCs. Antigens can also directly activate APCs through binding to TLR cell membranes. Inactivated viruses are phagocytosed by APCs, and traces of nucleic acid within the phagosome can activate endosomal TLR, leading to cytokine and chemokine production. After entering, the antigen is degraded in endocytic vesicles, then loaded onto MHC-II molecules and presented to CD4+ T cells. Activation of CD4+ T lymphocytes causes the production of cytokines and chemokines that induce the activation of antigen-specific B cells that mature into plasma cells that secrete antibodies and/or memory B cells. Whole- virus vaccines induce a potent humoral response and a low to moderate T cell response. CD8+ T cell activation occurs via an alternative pathway not depicted in this figure. The stimulation process in whole-virus vaccines is reported to be less frequent and less robust compared to viral vector vaccines (Jiang et al., 2020; Jam et al., 2025).

The effectiveness of a vaccine can be determined by its ability to withstand several variants that occur from the virus evolution process. Therefore, it is necessary to develop a vaccine platform that can adapt to many variants. To date, there is no data explaining the duration of immunity as an individual response after vaccination(Letko et al., 2020) . Several studies have reported a decline in immunity (*waning immunity*) to the Covid-19 vaccine. One study conducted by Levin et al. (2021) reported a decline in humoral immune response in individuals who had received a second dose of the BNT162b2 (Pfizer- BioNTech) vaccine in Israel, especially in men, among

people aged 65 years or older, and people with immunosuppression(Kai et al., 2021). Similar results were also reported from a study of healthcare workers in Belgium who had received two doses of the BNT162b2 (Pfizer-BioNTech) vaccine(Azkur et al., 2020), and several other locations. A study conducted in Qatar on participants in the Coronavac vaccine clinical trial showed a decrease in neutralizing antibody titers 6 months after the second dose of the vaccine(Cascella et al., 2020) . These results are similar to those reported in Thailand, which showed a decrease in antibody titers 3 months after two doses of Coronavac (Zhao et al., 2020).

The number of companies producing Covid-19 vaccines continues to grow, and they are beginning to introduce their products for global consumption, including in Indonesia. So far, most of the vaccines that have been distributed to the public appear to be effective and safe. However, the effectiveness of vaccination in protecting individuals and populations continues to be studied further. Information regarding the effectiveness of vaccines in immune response after Covid-19 vaccination in Indonesia is currently still being gathered. With immunity still declining, it is a concern whether the government needs to implement policies related to immunity status in the community. Knowing the immunity status of the community can provide further insight to help health authorities plan for future health system needs (Saif, 2020).

This study will evaluate the immune response formed after vaccination with two different types of vaccines, namely whole virus and viral vector vaccines. The immune response analyzed includes humoral and cellular immune responses. Studies related to immune responses in Indonesia are still very limited, especially regarding cellular immune responses to vaccines with whole virus and viral vector platforms. Even if there are studies, they are still limited to one type of vaccine and the observation period is not yet optimal, so they do not provide conclusions about the duration of the immune response after vaccination. Data collection will be carried out in the city of Bogor and Sleman Regency because both places already have the infrastructure to support cohort studies.

Research Method

This study was conducted in 2022-2023. Samples were collected in Bogor City for the group receiving the viral vector platform vaccine (AstraZeneca). Sampling was conducted on November 24-27, 2022, for a period of 12 months after the second dose of the vaccine was administered. The target population in this study was the group of people who received the AstraZeneca COVID-19 vaccine. The location or site used was the city of Bogor. The accessible population in this study was people who received the AstraZeneca COVID-19 vaccine who came to health care facilities (community health centers) or vaccination centers in the working area of the Bogor City community health center. The sample to be taken is the accessible population that meets the inclusion criteria to become a sample in this study and is willing to participate in the study by stating their consent on the informed consent (IC) form. The inclusion criteria for the sample include:

1. Willing to participate and sign *informed consent*
2. Age ≥ 18 years
3. Not pregnant
4. Not known or never confirmed positive for Covid-19
5. Have received the second dose of the AstraZeneca vaccine within 12 months before sample collection.

The sample size (n) to be used was calculated using the G power application for a paired two-group mean test. Based on the sample calculations that have been carried out, the minimum sample size used for the study is 78 samples for the virus vector vaccine (AstraZeneca) recipient group.

The data will be stored by the Research Team and only the Research Team will have access to it. Data generated from analysis using MacsQuant 10 flow cytometer software was then analyzed using SPSS 21.0. The data was first tested for normality using the Kolmogorov-Smirnov test. If the resulting P value was > 0.05, it meant that the data was normally distributed, and if the resulting P value was < 0.05, it meant that the data was not normally distributed. After the normality test, the data were analyzed using a paired two-group mean difference test (dependent

t-test) if the data were normally distributed. Otherwise, the Wilcoxon test was used. A P-value < 0.05 indicated significant results.

Discussion

From the target of a minimum sample size of 78, 90 blood specimens were successfully collected during data collection. On the day of data collection, the collected blood underwent laboratory testing, namely PBMC isolation, on the same day. PBMC isolation must be performed on fresh specimens. PBMC isolation was performed at the Genomics Laboratory of the National Research and Innovation Agency. The PBMCs obtained were then taken to Jakarta for temporary storage before undergoing cellular immune response testing using flow cytometry.

Although the objective of the study was to assess the cellular immune response after 12 months of the second dose, the government's policy of providing a third dose to the public meant that some subjects had already received a third dose. However, due to funding constraints, the cellular immune response analysis will still focus on research subjects who have only received the second dose.

Overall, the subjects involved in this study were mostly female (66.7%), aged 36-45 years (43.3%), and had a high school education (50%). In accordance with the government program that implemented a third-dose vaccination policy, some of the research subjects had received a third dose of the vaccine. A total of 5.67% had received the third dose of the vaccine, while the rest had only received the second dose. In terms of Covid-19 infection history, there was one research subject who had been exposed to SARS-CoV-2 infection.

Specific cellular responses were assessed using ex vivo stimulation of PBMCs with a pool of lyophilized peptides. PBMCs were added to *a 96-well plate* at a concentration of 1×10^6 in RPMI1640 supplemented with 10% human serum and then stimulated with 1 µg/ml PepTivator. For positive and negative controls, PBMCs were stimulated with 2.5 µg/ml Phytohemagglutinin-Latau or 2 µl sterile water with 10% DMSO, respectively. Cells were incubated for 20 hours at 37°C, 5% CO_2. After specific stimulation, cells were labeled with specific antibodies for flow cytometry analysis. Cryopreserved cells were thawed, washed, and stimulated for flow cytometry determination using cell marker assays. CD4 and CD8, B cells were analyzed after ex vivo stimulation of PBMCs with PepTivator for 20 hours. Data were analyzed using Kaluza Analysis Software.

Table 1. Characteristics of study subjects

Characteristics	Sample Size (N)	%
Gender Gender		
- Male	30	33.3
- Women	60	66.7
Age group (years)		
- 17 – 25	6	6.7
- 26–35	5	5.6
- 36–45	39	43.3
- 46–55	32	35.6
- 56–65	8	8.9
- 65 and above	0	0
Education		
- Did not complete elementary school/MI	4	4.4
- Elementary school/MI graduate	15	16.7
- Completed junior high school/MTS	25	27.8
- High school/MA graduate	45	50
- Completed D1, D2, D3	0	0
- Completed S1, S2, S3	1	1.1
COVID-19 vaccination status		
- 2 doses	39	43.3
- 3 doses	51	56.7
Brand of third dose vaccine		
- AstraZeneca	44	86.27
- Pfizer	7	13.72
Have you been infected with Covid in the past year?		
- No	89	98.8

Table 2. Average percentage of CD4+ and CD8+ T cell measurements

Variable	Percentage
CD4	70.00
CD8	70.00

In this study, data collection and analysis were

conducted 12 months after the second dose of both types of vaccines. The immune response formed in the body, both post-infection and post-vaccination, serves to prevent or reduce infection through the mechanisms of pathogen diffusion prevention, virus replication neutralization, bacterial opsonophagocytosis, and complement activation. Antibodies can last for some time depending on the type of vaccine, adjuvant, generality, and administration schedule. Live vaccines or virus particles have a longer duration. The presence of memory B cells is important in vaccination programs, so strategies are needed to maintain them. Secondary doses or boosters or the use of adjuvants play a role in inducing memory B cells, including the interval between booster doses, which affects memory B cell affinity.

When examining the characteristics of the samples from this study, they exhibit nearly identical variability. This is important because homogeneous samples provide valid and accurate measurement results. This indicates the occurrence of a decrease in antibody titers 12 months after the second dose of vaccination, as previous studies showed that four weeks after the second dose, the proportion of positive antibodies reached 100%. Previous studies also showed that 68.5% of subjects had positive titers before receiving the first dose of the vaccine, meaning that some subjects already had antibodies even though they had never received the Covid-19 vaccine.

Immunity was likely acquired through exposure to infection in the surrounding environment, as Covid- 19 cases were high in Indonesia at that time. After the first dose of vaccination, the proportion of positive titers continued to increase in subsequent observations at H14, H28, and reached 100% at H56 or four weeks after the second dose. For the AstraZeneca vaccine, the proportion of positive antibody titers remained at 100% twelve months after the second dose, showing no decrease compared to H112 or four weeks after the second dose.

When looking at antibody titers 12 months after the second dose of vaccination, the results are quite good. When compared to the median titer in previous studies, the median titer, which was originally 8985.05 AU/mL at H14 after the first dose, decreased fourfold to 2221.9 at H84 and increased slightly four weeks after the second dose (H112) to 3651.15 AU/mL. After 12 months, the antibody titer decreased to 2609.7 AU/mL (before receiving the third dose) and 5000.4 AU/mL (after receiving the third dose). A fairly high proportion of seropositivity (100%) after 12 months of the second dose of vaccination and the median antibody titer, especially for the AstraZeneca vaccine, is also supported by the results of a COVID-19 serosurvey conducted by the Ministry of Health in March 2022 across 34 provinces in Indonesia, which showed that 99.8% of the Indonesian population had antibodies against COVID- 19 with sufficiently high titers, regardless of their vaccination status.

The AstraZeneca two-dose vaccine clinical trial in the UK in the 18+ age group showed seropositivity in all groups 28 days after the second dose. The median antibody titer in each age group was sufficiently high: 18–55 years (20,713 AU/mL), 56–69 years (16,170 AU/mL), and ≥70 years (17,561 AU/mL). After the booster dose, the neutralizing antibody titer in all three age groups did not show a significant difference and reached >99.9%, as only one of 209 subjects had a negative neutralizing antibody titer. The results of this study indicate that although the AstraZeneca vaccine (ChAdOx1 nCoV-19) has the same immunogenicity in all adult age groups, it appears to be more tolerable in older adults than in younger adults, based on the safety testing of the vaccine.

Neutralizing antibody titers are very important because they can be used to predict protection against Covid-19 infection, especially symptomatic cases. Antibody titers usually decrease over time, but can increase if there is sufficient exposure to cause antibodies to rise again. Based on research in Germany conducted on medical personnel who had a history of Covid-19 infection and some of whom had received boosters, it was found that IgG antibody titers decreased slowly, but booster administration significantly increased antibodies. The increase in antibody titers due to booster vaccination was higher than that due to infection. However, the role of memory T cells was not correlated with the level of antibodies formed.

In our study, although seropositive antibodies 12 months after two doses of vaccination decreased slightly compared to before, the median titer

increased in subjects who had not received a booster. One possibility is exposure to Covid-19 infection in their surroundings, even though it did not cause significant symptoms, as only 1.8% of subjects reported having been infected with Covid-19 in the past year. Regarding booster vaccines, more than half of the subjects had received a booster vaccine. This is because since January 12, 2022, the government has recommended booster vaccines to complement the two doses of vaccine that had been given previously. In accordance with the Ministry of Health Circular Letter No. SR.02.06/II/1188/2022, there are currently four types of vaccines used in Indonesia, namely AstraZeneca, Pfizer, Moderna, and Sinopharm. However, in practice, the type of booster vaccine is adjusted to the availability of vaccines in the region.

T cell-mediated immunity plays an important role in the host's defense against infection, especially CD4+ T cells of the T-helper 1 (Th1) type that secrete interferon gamma (IFN-γ). CD8+ T cells can also produce IFN-γ to help destroy microorganisms. CD4+ T cells alone are not sufficient to control the growth of microorganisms; CD8+ T cells are also needed to increase IFN-γ production.6 Interferon gamma is the cytokine that plays the most important role in the host's defense against infection. Increased IFN-γ production by CD4+ T cells and CD8+ T cells is expected to protect against infection.

Decreased function of CD4+ T cells and CD8+ T cells in producing IFN-γ can interfere with the process of eliminating microorganisms. Failure of the immune response to eliminate and inhibit the replication of microorganisms that infect macrophages and dendritic cells in the alveoli. CD4+ T cells and CD8+ T cells have the same capacity to produce IFN-γ. Low levels of IFN-γ expression in CD4+ T cells and CD8+ T cells result in an unprotective immune response to infection.

Conclusion

The subjects involved in this study were mostly women aged 36-45 years who had received a third dose of vaccine (booster), had not been infected with Covid-19 during the past year, and showed signs of prehypertension based on blood pressure measurements at the time of sampling. Blood samples from patients have undergone PBMC isolation, and currently, only a portion has been analyzed for cellular immune response using flow cytometry. Preliminary results of the cellular immune response analysis show that the percentage of CD4+ and CD8+ T cell measurements is around 70%. Antibody titer and neutralizing antibody testing are required to predict protection against the current Covid-19 infection.

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