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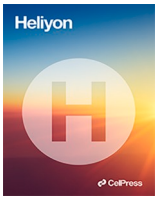

Inactivated SARS-CoV-2 vaccine candidate immunization on non-human primate animal model: B-cell and T-cell responses immune evaluation

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A R T I C L E I N F O

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A B S T R A C T

Background: SARS-CoV-2 vaccine was proven to be an effective and efficient measure for mitigating pandemic. COVID-19 infection and mortality subsided along with the increaseing COVID-19 vaccination coverage. Vaccine and health resource equity are predominant factors in COVID-19 pandemic management. Vaccine development for Indonesia, aims to ensure a sustainable pandemic control and steady national stability restoration. A decent vaccine must induce immunity against COVID-19 with minimum adverse reaction. Immunogenicity and ability to induce neutralizing antibody evaluation needs to be performed as part of the SARS-CoV-2 inactivated vaccine development from East Java, Indonesia isolate (*Vaksin Merah Putih-INAVAC*).
Objective: This research demonstrated INAVAC performance in inducing the production neutralizing antibody along with its effects on $CD4^+$ and $CD8^+$ cells response in *Macaca fascicularis* (non-human primate).
Methods: Two dosages of 3 μg and 5 μg were tested, compared to sham (NaCl 0.9%) in 10 *Macaca fascicularis* (2 injection intramuscular with 14 days interval). All animals were monitored daily for clinical signs. Nasopharyngeal samples were analyzed using qRT-PCR while the serum were tested using ELISA and neutralization assay, whereas PBMCs were flowcytrometrically analyzed to measure $CD4^+$ and $CD8^+$ population.
Results: It is observed that both vaccine doses could stimulate relatively similar immune response and neutralizing antibody (end GMT post challenge = 905,1), whereas higher $CD8^+$ cells response were reported in the 5 μg group after the 3rd day post-challenge. The dose of vaccine that produce adequate immune cell stimulation with neutralizing antibody induction can be adopted to clinical study, as favorable result of these parameters could predict minimum adverse reaction from inflammation response with balanced immune response.
Conclusions: Therefore, it is concluded that *Vaksin Merah Putih-INAVAC* with 3 μg dose showed a favorable potential to be developed and tested as human vaccine.

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1. Introduction

The most reliable specific prevention strategy to mitigate pandemic and the cornerstone of managing infectious disease outbreaks are vaccinations [1]. History of vaccination with smallpox has showed its capability for eradicating an infectious disease that harm humanity. Since Coronavirus Disease-2019 (COVID-19) pandemic was declared by World Health Organization (WHO) in March 2020, change in every dimension of human activity all around the world has been occurring. As of 7 December 2022 it is estimated that 641, 487,094 case and 6,621,419 death were reported [2]. Management of COVID-19 pandemic could also adopted similar approach. There were several reported vaccine development by WHO from all around the world [3]. Vaccine developments were also accelerated due to the pandemics that gave rise to adaptation of novel vaccine platforms including adenovirus-based platform, Deoxyribonucleic Acid (DNA) vaccine, and Messenger Ribonucleic Acid (mRNA) vaccine [4]. However, inactivated vaccine and subunit vaccine as older established vaccine technologies still exhibit its decent potency in combating COVID-19 pandemics [5].

To combat severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), Research Center for Vaccine Technology and Development-*Universitas Airlangga* (RCVTD-UNAIR) formulated chemically inactivated whole virus vaccine adjuvanted with Aluminium Hydroxide [6]. As the end of June 2022, a total of 201 million individuals in Indonesia had received first dose of vaccination and almost 170 million of individuals were fully vaccinated [7]. Nevertheless, few studies vaccine recipients focused largely on binding and/or neutralizing antibodies (NAbs) as primary endpoints, while vaccine-induced cellular immune responses remain elusive [8]. It is well established that three fundamental components of the adaptive immune system (B cells, $CD4^+$, $CD8^+$ T cells, and NK Cells) are essential to combat SARS-CoV-2 infection [9]. Despite the immune response and activation, the correlation towards COVID-19 protection remains unknown [10]. However, based on the last reported analysis of the COVID-19 immune response, it is reported that proliferation of RBD specific $CD4^+$ cells that include memory cells were responsible for neutralizing antibodies [11]. Antibodies production and T-cell responses are important for the prevention and resolution of primary SARS-CoV-2 infection, either through virus neutralization or cell cytotoxicity contributing to survival towards COVID-19 [12,13]. Additionally, SARS-CoV-2 infection and immunization induced various immunological memory components displaying distinct kinetics [14].

Immune system activation of signalling consists of several cascade since recognition of antigen by local immune cells, presentation towards presenting cell, non-specific immune response mechanism, and eventually followed by specific immune response mechanism [14]. Crosstalk of immune cells in inducing immune response involved several cytokines and chemokines related to inflammatory and anti-inflammatory response. Inflammatory cytokine consist of IL-6, TNF-α, IFN-γ and anti-inflammatory cytokine consist of IL-4, IL-10, IL-13. A balanced immune response could induce production of specific neutralizing antibody and immune cytotoxic cells without harming the host [15,16]. With sufficient immune activation towards vaccine, a decent immune response could be developed and hopefully protects the host from SARS-CoV-2 infection. For achieving decent immunity, a specific dose and interval must be investigated. It is reported that most sera elicited by two-dose were capable of neutralizing SARS-CoV-2 effectively, based on the *in vitro* assay [17]. Therefore, a comprehensive analysis of immune response towards vaccine and immunity towards SARS-CoV-2 could be measured and a benchmarking could be performed to ensure a decent vaccine performance. Several vaccine efficacy study from all around the world were reported by various vaccine developers [10].

In this current study, we provided data from this cohort with new insights into the kinetics of RCVTD SARS-CoV-2 inactivated vaccine-induced humoral and cellular immune responses, including circulating antibodies (antigen-specific B cells response), $CD4^+$ and $CD8^+$ T cells, at six timepoints extending up to 4–5 weeks during two-dose intramuscular immunization on non-human primate animal model (*Macaca fascicularis*). The impact of vaccine doses on the magnitude of vaccine responses were further analyzed. The interrelationships between antibody and cellular responses were also evaluated.

2. Methods

This phase-2 preclinical study of inactivated SARS-CoV-2 vaccine (*Vaksin Merah-Putih* or INAVAC) was the continuation of phase-1 preclinical study to explore the potential of SARS-CoV-2 local isolate inactivated vaccine.

2.1. SARS-CoV-2 inactivated vaccine

SARS-CoV-2 inactivated vaccine was formulated with 3 μg and 5 μg of SARS-CoV-2 inactivated antigens with 0.3 mg and 0.5 mg of Aluminium Hydroxide adjuvant respectively (volume 0.5 ml). Purification was performed through filtration, chromatography, and tangential flow filtration. SARS-CoV-2 inactivated antigens was produced by RCVTD, Institute of Tropical Disease (ITD), *Universitas Airlangga* (East Java Isolate; GISAID hCoV-19/Indonesia/JI-RSDS-RCVTD-UNAIR-35-A/2020) on Vero Cell host [6].

2.2. Macaca fascicularis

The animal experiment was designed and performed according to the principles in the "Guide for the Care and Use of Laboratory Animals" and "Guidance for Experimental Animal Welfare and Ethical Treatment" [18,19]. The protocols were reviewed and approved by Faculty of Veterinary Medicine Ethical Committee, Airlangga University (reference number: 2.KE.118.03.202). All animals were fully under the care of veterinarians. *M. fascicularis* was retrieved from PSPP, Bogor, Indonesia. A total of 10 *M. fascicularis* (5 male and 5 female) were randomly assigned to control and vaccination group. Vaccination group consisted of 1st group that received 3 μg vaccine dose (2 male and 2 female), 2nd group that received 5 μg vaccine dose (2 male and 2 female), and control group (sham) that

received NaCl 0.9% (1 male and 1 female). *M. fascicularis* were quarantined and nurtured in Airlangga University Animal Biosafety Level-3 (ABSL-3) facilities. Animal acclimatization was performed for 14 days. *M. fascicularis* diet consisted of fruit and dry food with water ad libitum.

2.3. Immunization and blood collection

Immunization procedure of *M. fascicularis* was performed by intramuscular injection of 0.5 ml of vaccine on left deltoideus muscle. Immunization was performed twice with 14 days interval. *M. fascicularis* weight, temperature, and behaviour were observed. Blood collection was performed by venepuncture in femoral vein by trained veterinarian after sedation with ketamine (10 mg/kg intramuscular) [20]. Blood samples were collected in plain vacuum container and serum was separated and stored in −80 °C deep freezer, whereas PBMCs (Peripheral Blood Mononuclear Cells) were collected using Lymphoprep (STEMCELL Technologies) gradient centrifugation from blood stored in heparinized vacuum container [21].

2.4. Viral challenge

Macaques from control and vaccination group were infected with SARS-CoV-2 (2×10^5 $TCID_{50}$/macaque) intratracheally according to the requirements of ABSL-3 [10]. All animals were monitored daily for clinical signs. Nasopharyngeal secretions and blood samples were obtained at 3, 5, and 7 dpi (days post infection). Blood specimen was collected under appropriate anaesthesia with ketamine (10 mg/kg IM) to alleviate pain and minimize suffering. Two macaques from the 3 μg, 5 μg dose vaccine group were euthanized at 3, 5, and 7 dpi, whereas one macaque from control group was euthanized at 7 dpi. The remaining 3 macaques were used to observe clinical manifestations and viral shedding (see Fig. 1).

2.5. In-house ELISA antibody measurement

Separate microwells of 96-well ELISA plates (NEST, China) were coated with purified inactivated viral antigen at a concentration of 2 μg/100 μl/well and incubated at 4 °C overnight [22]. Then, the plates were blocked with 5% BSA in phosphate-buffered saline (PBS), incubated with serially diluted serum samples (1 h, 37 °C). Three times wash was performed using PBS-T after incubation. HRP-conjugated anti monkey IgG Secondary antibody was added (1:1000; 1 h; 37 °C). After secondary antibody incubation the plate was washed 3 times with PBS-T. For visualization, 100 μl TMB substrate (Biolegend, San Diego, California) was added to each reaction wells and then incubated for 15 min in room temperature). The reaction was stopped using 100 μl 2 M H_2SO_4 solution. The absorbance of each well was measured at 450 nm in an ELISA plate reader (Thermo Scientic Multiskan EX, Waltham, Massachusetts, U.S.), and the following equation was used: resulting OD (optical density) = (experimental well OD) – (mock well OD). The pre-immunization sera of animals were used as the negative controls. ELISA optimization was performed by linear analysis of antigen and positive serum titration with R^2 value ≥ 0.95. The GMT was calculated as the geometric mean of the endpoint titers of positive sera in the same group [23].

2.6. Neutralizing antibody test

Heat-inactivated serum from macaques was serially diluted and co-incubated with live virus (100 $TCID_{50}$/well) for 1 h at 37 °C. Then, 100 ml of the Vero cell suspension (10^5 cells/mL) was added to the mixture before seeded to 96-well microplates with addition of methylcellulose MEM. The plates were then incubated at 37 °C in a 5% CO_2 atmosphere for 3 days. The mediums were discarded and the cells were stained with crystal violet solution before washed 3 times with PBS. The CPEs were observed and assessed with an inverted microscope (Nikon, Japan) to determine the neutralizing antibody titer of the serum [23,24].

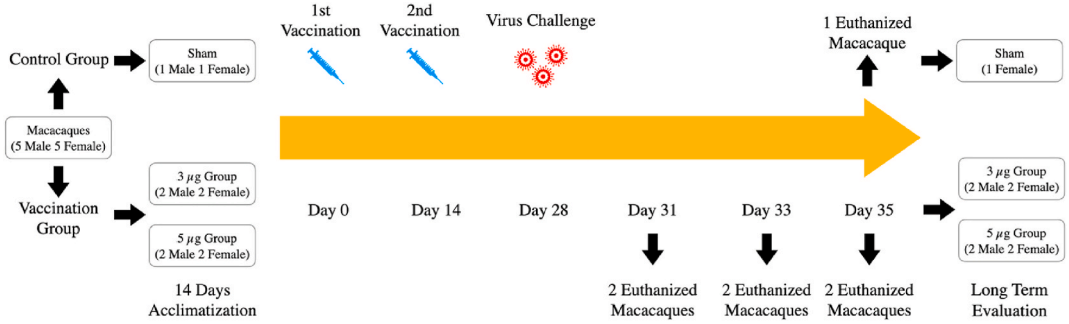

Fig. 1. Study Timetable (Day 0: day of first vaccination; Day 14: second vaccination; Day28: Viral Challenge; Day 31: 3 days post infection; Day 33: 5 days post infection, Day 35: 7 days post infection). Blood samples were obtained from each timepoint and nasopharyngeal swab specimens were collected at Day 31, Day 33, and Day 35.

2.7. Immune cell populations analysis

PBMCs were isolated from macaques using a lymphocyte isolation technique (Lymphoprep™; Stem Cell Technologies) [21]. Anti-human CD4 FITC-conjugated, and anti-human CD8 PE-conjugated were added to the PBMCs in PBS. The mixtures were incubated at room temperature for 30 min in the dark. Reagents for red blood cell lysis (Cat# 349202; BDIS) and membrane permeabilization (Cat# 554714; BD Biosciences) were added sequentially. After two washes with PBS, the cells were resuspended in PBS and detected using a flow cytometer (FACScalibur, BD, USA). $CD4^+$ T Helper Cells, and $CD8^+$ Cytotoxic T Cells were evaluated [25].

2.8. Nasopharyngeal swab qPCR

Nasopharyngeal swab was performed using paediatric nasopharyngeal swab and immediately storage on virus transport media. RNA extraction was performed using QIAamp Viral RNA Mini Cat. No./ID: 52904 (Qiagen), according to supplied protocols. qPCR was performed using Allplex 2019-ncov assay^{tm} (Seegene, South Korea) from RNA extracted nasopharyngeal swab specimen before *M. fascicularis* vaccination, pre-challenge, and post-challenge [26].

2.9. Statistical analysis

Data analysis was performed by analysing data distribution through Kolmogorov-Smirnov analysis. The data are presented as the means and standard deviations (SDs) for normally distributed data and median with minimum and maximum for non-normally distributed data. IBM SPSS Statistics 26 software (USA) was used for statistical analyses. Independent Sample T-test was performed to compare the difference between the two groups with one factor. Two-way ANOVA was performed to compare the differences between the groups with multiple factors. The association of antibodies was determined using Spearman rank correlation analysis. Parametric analysis was performed in normally distributed data and non-parametric was performed in non-normally distributed data [27].

3. Results

3.1. Anti-SARS-CoV-2 antibody response in M. fascicularis

It was identified that pre-vaccination ELISA OD_{450} or baseline were 0,154 ± 0,031 as shown in Fig. 2 with similarity across vaccination and sham group ($p = 0.057$; ANOVA). OD_{450} increase after 14 days post initial vaccination were detected on 3 μg and 5 μg group ($p = 0.949$). After 14 days post second dosage a higher OD_{450} were clearly detected with comparable OD_{450} between 3 μg and 5 μg group ($p = 0.625$). Steady increase of OD_{450} was observed after virus challenge. There were relatively unchanged ELISA OD_{450} in sham group. There was insignificant difference between OD_{450} between 3 μg and 5 μg group in each observation (see Fig. 2).

3.2. M. fascicularis neutralizing antibody titer

The result of Plaque Reduction Neutralization Test (PRNT) was shown in Table 1. Based on the PRNT it is verified that there was no neutralization activity in pre-vaccinated serum. There was moderate neutralizing activity of the serum in vaccine group after first vaccination. After second dosage administration it is detected that highest neutralization capacity was detected on the 3 μg group and the least in 5 μg group. After challenge test the neutralization performance were also maintained with similar neutralization capacity.

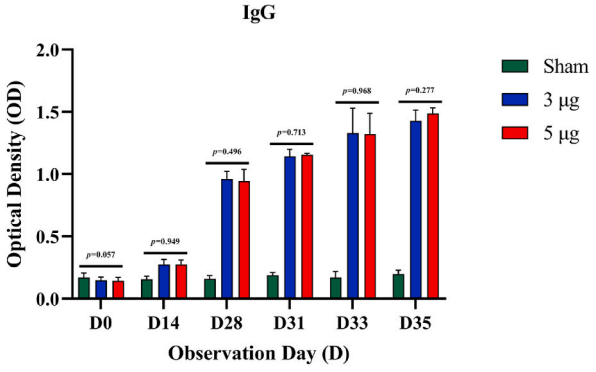

Fig. 2. *Anti*-SARS-CoV-2 Antibody level based on ELISA OD_{450}.

Table 1
M. fascicularis neutralizing antibody titer plaque reduction neutralization test (PRNT).

Observation Point	3 μg		5 μg		Sham	
	$TCID_{50}$	GMT	$TCID_{50}$	GMT	$TCID_{50}$	GMT
D14	1/80	63.50	1/80	50.40	n/a	n/a
	1/80		1/40		n/a	n/a
	1/40		1/40		n/a	n/a
D28	1/640	639.99	1/80	403.17	n/a	n/a
	1/1280		1/1280		n/a	n/a
	1/320		1/640		n/a	n/a
D31	1/1280	n/a	1/640	n/a	n/a	n/a
D33	1/640	n/a	1/1280	n/a	n/a	n/a
D35	1/640	905.10	1/1280	905.10	n/a	n/a
	1/1280		1/640		n/a	n/a

3.3. $CD4^+$ cells population response

Flowcytometry analysis of $CD4^+$ cells population in *M. fascicularis* PBMCs suggest that the level of $CD4^+$ cells in vaccination group was comparable to sham group in vaccination phase (D0-D28) as shown in Fig. 3. During virus challenge it is also observed that $CD4^+$ population were relatively unchanged (Day 31-Day 35).

3.4. $CD8^+$ cells population response

After first vaccination the $CD8^+$ cells in PBMCs were comparable to the sham group. Evaluation post-second vaccination also showed similar findings. However, an increase of $CD8^+$ cells population were detected after challenge predominantly during D3 post infection (D31). The highest increase of $CD8^+$ cells population was observed in 5 μg group compared to sham, whereas there was similar $CD8^+$ response in 3 μg group compared to sham group. During D33 and D35 the level of $CD8^+$ percentage became relatively insignificance among vaccinated and sham group (see Fig. 4).

3.5. Nasopharyngeal swab qPCR

In the beginning before experiment was performed and after vaccination, the entire *M. fascicularis* were negative for SARS-CoV-2 qPCR. Then after virus challenge, there were no *M. fascicularis* in vaccine group that positive for SARS-CoV-2 qPCR. However, The entire sham group *M. fascicularis* was positive for SARS-CoV-2 qPCR.

4. Discussion

COVID-19 vaccination as a specific measure for pandemic mitigation has shown its efficacy and potency by reducing COVID-19 case, severity, and mortality [1]. Various technologies were also adopted for vaccine development. DNA and RNA vaccine with vector virus vaccine are a novel platform of vaccine that approved and showed its efficacy during pandemic progression [4]. However long-established protein subunit and inactivated vaccine also display its decent efficacy comparable to the new-fashioned platform [5]. Besides specific prevention measure, an efficient and evenly distributed health resource must also be performed to ensure equity of healthcare all around the world, as pandemic is a global issue [28–30]. Eradication of potential SARS-CoV-2 locus or sanctuary globally could eventually lead to well controlled disease and hopefully could end COVID-19 pandemic [31].

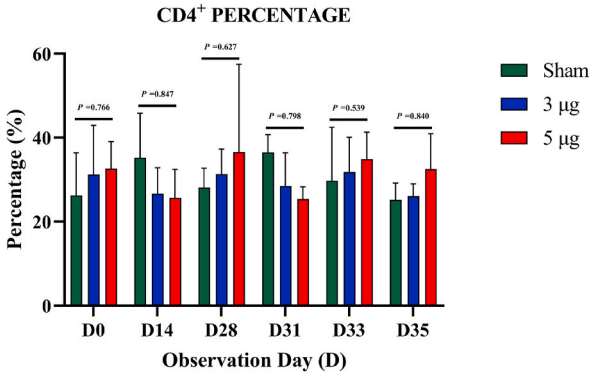

Fig. 3. $CD4^+$ population percentage.

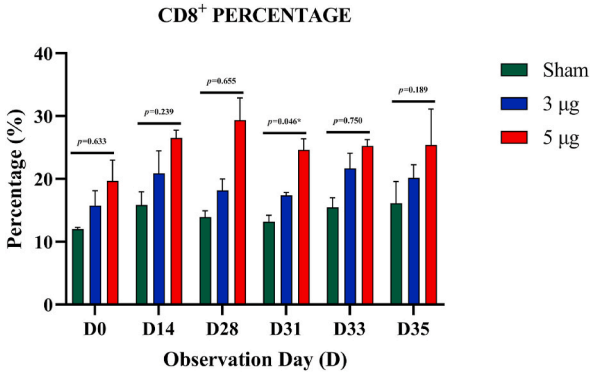

Fig. 4. $CD8^+$ cells population percentage.

Healthcare equity for the pandemic mitigation could face several issues regarding transportation limitation, limited resource, and limited personnel [32]. Transportation limitation during pandemic has shown its effects by delayed supply of various resources [33]. Delayed transportation and logistics further affect economy and a wider range of human life. Personnel limitation for resource manufacture and healthcare provider aggravate pandemic mitigation effort, however a management of limited human resource must be performed [32]. A triage procedure and effective utilization of limited health resource could help alleviate this issue. Limited resource such as mask, drugs, and vaccine could also worsen pandemic progression [34]. These key problems could render, even a developed country, face government, economy, and social major problems. In developing country point of view a pandemic brought more complicated issues [35].

Focused on limited health resource, vaccine development and production were mainly performed by resource capable countries [3]. For ensuring national supply, a steady and adequate production capacity must be maintained, and after national demand is fulfilled, international demand could be fulfilled. There were several vaccine development and production that were reported to WHO during the course of pandemics [3,28,30]. Developing countries were also developing and producing COVID-19 vaccine, this development could ensure a steady pandemic mitigation effort. Indonesia as a developing country with fourth largest population in the world must also perform this step to ensure national stability [36]. Therefore, a development of inactivated SARS-CoV-2 vaccine was performed [6]. Exploration of SARS-CoV-2 East Java isolate further analyzed and formulated to inactivated vaccine, with additional data of B cell epitope prediction study data that suggesting a relatively decent potency as a vaccine candidate compared to newer circulating SARS-CoV-2 variant [37]. It is reported that in 2023 SARS-CoV-2 variant in Indonesia mainly composed of omicron variant with domination of XBB and BA lineage [38]. This vaccine performance should be tested in animal models and then in clinical trial for objective evaluation of its protective potency [10].

Macaca fascicularis as non-human primate animal model was proven to be able to mimic human metabolism and immune response [21]. To ensure immunogenic properties of vaccine an animal trial must be performed. In this trial we performed preclinical trial that involved male, female, elder, and young *Macaca fascicularis* as model for heterogenous age group population. Intramuscular route of vaccination was performed to use 'depot effects' of muscle tissue, mechanism that involved the slow release of antigens from the site of immunization. A dose of 5 ml injection was used as the muscle volume is comparable to human. A dosage of 5 µg and 3 µg of antigens was used to provide a comparable level to previous reported findings [10].

As the purified inactivated whole SARS-CoV-2 antigen was used, an immune response towards its proteins was predicted, therefore in this study an in-house ELISA protocol was performed to assess *anti*-SARS-CoV-2 IgG response. In this study it is observed that after first vaccination there were unnoticeable level of OD_{450} among vaccinated and sham group. This data suggests that during first antigen exposure an immune response triggers production of IgM and followed by IgG with declining level of IgM [39]. After second dosage of vaccination, an apparent increase of OD_{450} could be observed in vaccination group compared to sham group. *Anti*-SARS-CoV-2 IgG OD_{450} response was observed to be relatively similar among 3 µg and 5 µg group. Similar and comparable response in towards 3 µg and 5 µg antigens suggest that 3 µg dosage could be adapted as vaccination dose as a cost effective and efficient approach similar to previous report [10,40,41]. Besides IgG levels as a measured variable of immunogenicity, a neutralization test must also be performed to assess its efficacy. IgG produced from vaccination tend to be heterogenous and not every IgG possess neutralization activity towards viruses [42,43]. Therefore, PRNT analysis should be performed.

PRNT analysis in this study showed that after first vaccination there were neutralization activities exhibited by immunized serum, and a higher titer of neutralizing antibodies was detected after second vaccination. A comparable neutralization capacity was observed among 3 µg and 5 µg group. No neutralization activity was observed in sham group and pre-vaccinated serum. These findings confirm SARS-CoV-2 inactivated vaccine candidate potency of East Java isolate [6]. Analysis of immune activation could also be performed to confirm these findings and might provide information in predicted inflammatory related adverse reaction [44].

Immune cells activation could be detected by assessing several immune cells that plays important role in immune response especially $CD4^+$ T helper cell, and $CD8^+$ Cytotoxic T Cells. Immune cells in the circulation could be detected in PBMCs through flowcytometry analysis with fluorescent tagged antibody could be enacted [11,44]. $CD4^+$ cells population showed a relatively similar response after first and second vaccination compared to sham group during vaccination and after virus challenge. In this research with

the usage of inactivated vaccine formulated with aluminium hydroxide adjuvant, it is predicted that predominant focused on $CD4^+$ cells response [45,46]. It is reported that antigen specific $CD4^+$ cells is needed for the production of neutralizing antibody [47], and limitation in this study only measured unspecific $CD4^+$ cells population.

On the other hand, $CD8^+$ cells population were also showed similar response after first and second vaccination compared to sham group however, after virus challenge (D31) a significant increase of $CD8^+$ was detected in 5 μg group. Then, $CD8^+$ cells response became relatively comparable in D33-D35 among vaccinated and sham group. These findings suggest that immune activation towards inactivated vaccine with alum adjuvant existed in different pole compared to live virus infection [48]. Immune response towards infection of SARS-CoV-2 live virus, a tissue damage and cell death that occurs after virus infection could trigger cell mediated immune response through cytotoxic T cells activation (including $CD8^+$ cells subset) and mobilization cell mediated immunity to kill infected cells. However, this findings need more confirmation as this study analysis of $CD8^+$ cells were limited based on the total unspecific $CD8^+$ cells population. SARS-CoV-2 specific $CD8^+$ cells population towards analysis is needed to confirm if this response is related to antigens presented in the vaccines. It is also reported that $CD8^+$ cells response towards SARS-CoV-2 infection is host dependent and could be heterogeneous that might explain our findings [49]. Moreover, it is confirmed that SARS-CoV-2 inactivated vaccine predominantly induced $CD4^+$ cells response compared to $CD8^+$ [50]. Combined with antibody neutralization of live virus from destroyed cells, synergy of these immune response eventually could subside virus infection effectively. The immunity towards SARS-CoV-2 could be observed in milder symptoms and negative nasopharyngeal swab qPCR detection of SARS-CoV-2 [51,52].

Different response of $CD8^+$ cell count in 5 μg group could indicate a more $CD8^+$ related immune response towards live pathogens (SARS-CoV-2), as vaccine elicited immune response were mainly depend on the vaccine type and adjuvant. However, higher $CD8^+$ cells response might indicate a possible acute increase of cytokine and chemokine that linked to inflammatory response that might linked to vaccine adverse reaction and reactive inflammation [51,52].

The similar response in 3 μg group and sham group might indicate that $CD8^+$ immune response was not as robust in 5 μg group. The cause of this results might be related to circulating neutralizing antibody that could neutralize SARS-CoV-2 before infection could progress in the vaccinated group (3 μg), and the lack of immune memory towards SARS-CoV-2 in sham group which need more time for immune recognition [53]. It is reported that aluminium hydroxide induced predominantly, and relatively negligible cell mediated immune response [46]. However, different antigens and adjuvant combination could also affect host immune response. Based on the previous report in SARS-CoV-2 inactivated vaccine adjuvanted with alum, it is reported that these vaccine formulation were lack of $CD4^+$ and $CD8^+$ cells activation. Even with the similar $CD4^+$ and $CD8^+$ cells response, this vaccine formulation were able to induce SARS-CoV-2 neutralizing antibody that offers immunity [51,52]. Moreover, additional report of COVID-19 pathophysiology that involves cytokine storm, and immune cell exhaustion which are linked to unbalanced immune response that lead to mortality has been well established [54,55]. Therefore, it is concluded that a balanced immune response were required to induce protective immunity towards COVID-19 vaccination and ultimately protect patients from COVID-19 severe reaction and mortality.

5. Conclusion

Based on this data, vaccination of *Macaca fascicularis* with inactivated SARS-CoV-2 vaccine originated from East Java, Indonesia isolates could induce *anti*-SARS-CoV-2 antibody production and exhibit neutralizing properties with a relatively balanced $CD4^+$ and $CD8^+$ cells response (especially in the 3 μg dose). Therefore supported by this data, a scale-up pilot production and human trial could be performed to assess the adaptability of this vaccine prototype for clinical usage. Moreover, a more holistic approach also including safety, and immunocharacterization of this vaccine elicited immune response must also be performed to objectively profiling vaccine safety besides its efficacy.

Author contribution statement

Rofiqul A'la: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Andi Yasmin Wijaya: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Helen Susilowati and Suryo Kuncorojakti: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Diyantoro Diyantoro: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jola Rahmahani: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Fedik Abdul Rantam: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data associated with this study has been deposited at https://repository.unair.ac.id.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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