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IMMUNOGENICITY STUDY OF SARS-COV-2 NUCLEOCAPSID PROTEIN SYNTHETIC FRAGMENTS USING LOCAL RABBITS

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ABSTRACT

This study was carried out to analyze whether an insilico designed SARS-CoV-2 Nucleocapsid protein fragments could induce local rabbit immune response. A peptide fragment with the amino acid sequence VLYNSASFSTFKCYGVSPTKLNDLCFT, previously published elsewhere, was used as a synthetic vaccine. Two healthy local male rabbits were immunized according to standard immunization procedures. Each rabbit was immunized subcutaneously at several points on the dorsal with a dose of 100 µg/rabbit, while for a booster at a dose of 50 µg/rabbit at intervals of 2 weeks, until the third booster. Antibodies were obtained from the sera after third boosters, purified using 50% ammonium sulfate precipitation, and tested using dot blot and western blot techniques. This study shows succeed in inducing the local rabbit's immune response. The resulting antibody reacted to the designed peptide tested. Whether the antibodies obtained can be applied for immunodiagnostic development studies against SARS-CoV-2 antigens in the actual circumstances needs to be explored further.

Keywords: Synthetic peptide, vaccine, SARS-CoV-2, rabbit polyclonal antibody

1. INTRODUCTION

At the end of 2019 a new outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged, which was first isolated from three people in Wuhan, and then the virus was given the name COVID-19 [1]. SARS-CoV-2 is a member of the corona virus family that causes respiratory syndrome. COVID-19 has caused a global pandemic that has resulted in tens of thousands of infections and thousands of deaths worldwide. To date, there are no appropriate therapies or antiviral agents that are specific for SARS-CoV-2. Researchers are racing to find alternative life-saving treatments, thankfully in the midst of these difficulties; efforts to produce valuable vaccines for the prevention of SARS-CoV-2 infection have yielded encouraging results [2]. Although there are

already a number of successful COVID-19 vaccines worldwide, studies related to producing antibodies against SARS-CoV-2 for laboratory needs and diagnostic development purposes are still needed.

Corona virus uses a multi-subunit replication/transcription engine. A set of non-structural proteins (NSPs) produced as cleavage products of the viral polyproteins ORF1a and ORF1ab [3] combine to facilitate viral replication and transcription. The main component, RNA-dependent RNA polymerase (RdRp, also known as NSP12), catalyzes viral RNA synthesis and thus plays a central role in the replication and transcription cycle of the COVID-19 virus, thought to be with the help of NSP7 and NSP8 as co-factors [4].

The N protein is the only structural protein associated with the replicase-transcriptase complex and binds to genomic RNA in corona viruses [5]. This protein is multifunctional and is one of the most important structural components of the corona virus. The N proteins are structural and antigenic proteins involved in the packaging, transcription, and replication of corona viruses [6], hence N proteins are promising not only as a vaccine candidate, but also for the development of diagnostics to detect the presence of SARS-CoV-2.

The development of vaccine research shows that the use of biotechnology plays a very important role, both for recombinant and peptide vaccines. Patronov and Doytchinova [7] emphasized that peptides and epitopes have proven to be desirable candidates for vaccine development due to their relatively easy production, chemical stability, and lack of infectious potential. In recent years, the experimental design and production of multi-epitope vaccines has increased dramatically, including in the animal husbandry and veterinary world. Inducing an immune response and producing polyclonal antibodies against peptide fragments, designed in silico has been reported [8]. Furthermore Kisworo and Depamede [9] succeeded in designing immunogenic peptides using bioinformatics methods, while Wariata et al. [10] succeeded in designing an in silico synthetic peptide to produce polyclonal antibodies against the synthetic peptide Binder of Sperm-1 (BSp-1). The reports show that immunogenic peptides can be designed in silico and are capable of producing antibodies in vivo.

Enayatkhani et al. [11] has designed a novel multi-epitope vaccine against COVID-19 with a bioinformatics technology approach. Three known antigenic proteins (Nucleocapsid, ORF3a, and membrane protein, referred to as NOM) of the virus were selected and analyzed to predict the epitope potential of B cells and immunogenic cells and then validated using bioinformatics tools. According to them, from the results of the in silco analysis, the designed vaccine has the potential to trigger an immune response of CD4+ and CD8+ T cells. In this paper it is reported that we have successfully produced antibodies against a fragment of the designed NOM vaccine [11] in local rabbits.

2. MATERIALS AND METHOD

This study used synthetic fragments of peptide ordered from GenScript, with amino acid sequences derived and modified from [11]. The experimental animals used were two healthy adult male local rabbits. The treatment of experimental animals was in accordance to regulations at the Faculty of Animal Science, University of Mataram.
The vaccine preparation and vaccination process were carried out based on [8] with modifications.

a. Synthetic peptide residues from the NOM vaccine fragment, i.e. the selected N-protein, were chosen and modified into VLYNSASFSTFKCYGVSPTKLNDLCFT, and coupled to the keyhole limpet hemocyanin (KLH, Sigma Aldrich) as protein carrier. An amount of 10 mg of the fragment peptide was coupled with 3 mg of activated KLH using MBS (3-maleimidobenzoic acid N-hydroxy-succinimide ester, Sigma Aldrich) as a linking reagent.

b. The immunization process includes making an emulsion with complete Freud's adjuvant (CFA for the first immunization), and incomplete Freund's adjuvant (IFA for boosters). Each rabbit was immunized subcutaneously at several points on the dorsal with a dose of 100 µg/rabbit, while for a booster at a dose of 50 µg/rabbit at intervals of two weeks, until the third booster.

c. Purification and antibody testing. Serum antibody was harvested 10 days after the last booster and purified by ammonium sulfate precipitation method. The purified antibodies were then tested using dot blot and western blot methods against the vaccine coupled with bovine serum albumin (BSA) instead of KLH.

d. The data obtained were evaluated descriptively

3. RESULSTS AND DISCUSSION

This study aims to obtain antibodies against SARS-CoV-2 to study antigen-antibody reactions in immunodiagnostic development. The limited facilities for isolating antigens from native viruses led us to use synthetic peptides. In this study we used the SARS-CoV-2 peptide fragment designed by Enayatkhani [11], more specifically, we took the nucleocapsid (N protein) moiety. We also specifically selected local rabbits found in our area, as experimental animals. Due to the small size of the synthetic peptide fragment, before being immunized, it had been coupled with a courier protein, KLH [12]. The immune response was observed ten days

after the third booster using the dot blot method and the results are presented in Figure 1. From the two local rabbits used, it appears that there is a slight difference in the quality of the immune response descriptively between rabbit 1 and rabbit 2. Rabbit 2 appeared to produce a stronger response than rabbit 1, while pooled sera from non-vaccinated rabbits did not give immune respond, as shown in Figure 1, respectively. From this it appears that the peptide fragment vaccine used in this study succeeded in triggering the rabbit immune response.

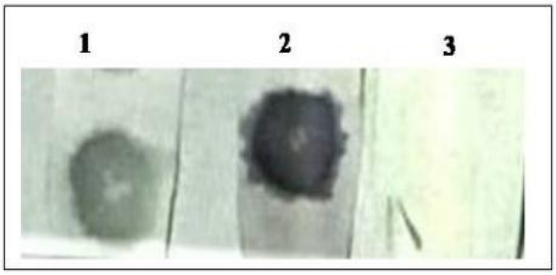

Figure 1. Dot blot test results of antibodies against COVID-19 protein-N synthetic peptide fragments. Nitrocellulose membrane was dotted with the BSA-coupled peptide then reacted with vaccinated (1 and 2) and unvaccinated rabbit serum (3).

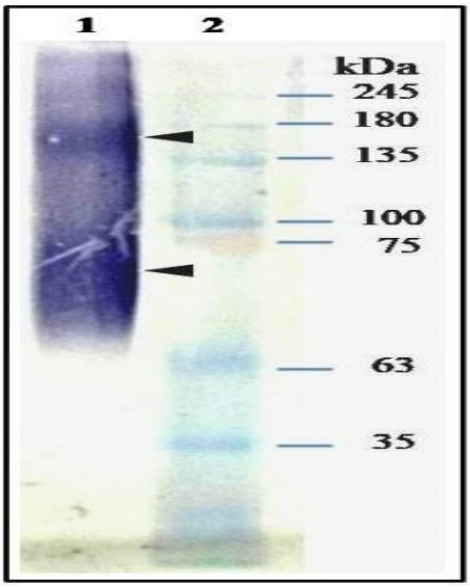

Figure 2. Western blot test results. The arrowheads (lane 1) indicate the immunogenicity of the antibody against the peptide fragments. Molecular marker (lane 2; GangNam-STAIN™, Prestained Protein Ladder, Intron).

Based on the results in figure 1, further testing was carried out using the western blot technique, and the results are presented in Figure 2. It can be seen that the majority response occurred to the two main bands of the protein-N synthetic peptide fragments coupled with BSA used in the assay. The splitting of the bands (arrowheads, Fig. 2) was probably due to the boiling treatment of the sample before it was applied to 10% SDS-PAGE in the western blot assay process. Furthermore, there was still a non-specific background on the nitrocellulose membrane; this was probably due to over enzymatic reactions. Other than that, it is highly probable that this was due to the antibodies used was only partially purified using 50% ammonium sulfate precipitation, or other factors [13].

Although there is still a non-specific background, putting together it is clear that in this study COVID-19 protein-N synthetic peptide fragments succeeded in triggering the local rabbit immune response, in the form of polyclonal antibodies. These results are in accordance with Enayatkhani et al. [11] that the peptide designed by them is immunogenic, capable of triggering B cells and T cells. At the time of this study, due to limited authority we did not perform immune assays using native- or recombinant proteins or with the intact peptide sequences. It would be better if such experiments be carried out in the near future hence it can be ascertained whether the antibodies obtained can be employed for immunodiagnostic development studies against SARS-CoV-2 antigens in the real situations.

4. CONCLUSIONS

In this study we succeeded in inducing local rabbit's immune response using the synthetic peptide VLYNSASFSTFKCYGVSPTKLNDLCFT, a fragment of the SARS-CoV-2 Protein N. The resulting antibody reacted to the target peptide tested using dot blot and western blot assays.

5. ACKNOWLEDGMENTS

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