Immunoinformatics Analysis of SARS-CoV-2 Isolated from Karst of Bats in Malang, Indonesia

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Abstract

In December 2019, cases of mysterious pneumonia were first reported in Wuhan, Initially, the disease was temporarily named as 2019 novel coronavirus (2019-nCoV), then WHO announced a new name on February 11, 2020, namely Coronavirus Disease (COVID-19) which is caused by a virus Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). This virus can be transmitted from person to person and has spread widely in China and more than 190 other countries and territories. Meanwhile in Indonesia, up to September 2020, 203 thousand cases of COVID-19 were found and 8336 deaths. Bats act as important disease reservoirs for various etiologic agents of disease that can be transmitted between species, infecting humans and mammals, both domestic and wild. The purpose of this study was to analyze SARS-CoV-2 immunoinformatics in bats in Karst Malang Indonesia as the basis for making SARS-CoV-2 vaccines. This research method is SARS-CoV-2 amino acid bats in Karst Malang, Indonesia analyzed by immunoinformatics. From the research results, it was found that SARS-CoV-2 protein from Malang Karst bats were antigen, non-allergen and non-toxin, so that they could be used as vaccine candidates, diagnostic kits and immunotherapy.

Keywords: *SARS-CoV-2, Bats, Malang Karst, Indonesia, Immunoinformatics.*

Introduction

In December 2019, cases of mysterious pneumonia were first reported in Wuhan, Hubei Province. The source of the transmission of this case is still uncertain, but the first case was linked to a fish market in Wuhan. From 18 December to 29 December 2019, five patients were treated with Acute Respiratory Distress Syndrome (ARDS). From 31 December 2019 to 3 January 2020, this case increased rapidly, marked by the reporting of 44 cases. In less than a month, the disease has spread to various other provinces in China, Thailand, Japan and South Korea. The samples studied show the etiology of the new coronavirus.2 Initially, the disease was temporarily named as 2019 novel coronavirus (2019-nCoV), then WHO announced a new name on 11 February 2020, namely Coronavirus Disease (COVID-19) caused by the Severe Acute Respiratory Syndrome Virus Coronavirus-2 (SARS-CoV-2). This virus can be transmitted from person to person and has spread widely in China and more than 190 other countries and territories.5 On 12 March 2020, WHO announced COVID-19 as a pandemic. As of 29 March 2020, there were 634,835 cases and 33,106 total deaths around the world. Meanwhile in Indonesia, up to September 2020 there were 203 thousand positive cases of COVID-19 and 8336 cases of death[1,2,3].

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Bats are classified as the Chiroptera Nation, and are the only mammals that have the ability to fly properly using wings[4]. The number of bats is more than 1200 species around the world. These small mammals occupy the second largest number of rodents (Rodentia) in the Mammalia Class[5]. There are 215 types of bats scattered throughout Indonesia[6]. Fruit-eating bats (Pteropodidae family) are bats that consume fruit and / or flower products, ecologically they play an important role in nature as seed dispersers and flower pollinators for certain plants[7]. Pteripodidae also act as important disease reservoirs for various etiologic disease agents that can be transmitted between species and infect humans and mammals, both domestic and wild. Various types of important viruses can be transmitted by Pteropodidae, including Henipavirus[8], Lyssavirus[9], Coronavirus associated with SARS and MERS[10,11] and Filovirus (including the Ebola virus. and Marburg virus)[12]. Various efforts have been directed towards the development of the COVID-19 vaccine, to prevent a pandemic and most of the developing vaccine candidates have used the S-protein SARS-CoV-2[13]. As of 2 July 2020, the worldwide SARS-CoV-2 vaccine landscape includes 158 vaccine candidates, of which 135 are in the preclinical or exploratory stages of development. Currently, mRNA-1273 (Moderna), Ad5-nCoV (CanSino Biologicals), INO-4800 (Inovio, Inc.), LV-SMENP-DC, Pathogen-specific aAPC (ShinzenGeno-Immune Medical Institute), and ChAdOx1 (University of Oxford) has entered phase I / II clinical trials (WHO, 2020). Vaccines that are in the channel are based on live inactivated or attenuated viruses, protein sub-units, virus-like particles (VLP), virus vectors, DNA, RNA, nanoparticles[14]. To improve immunogenicity, various adjuvant technologies such as AS03 (GSK), MF-59 (Novartis), CpG 1018 (Dynavax)[15]. An immuno-informatics approach was also used to identify the epitopes of the SARS-CoV-2 vaccine candidate. It can be used to identify significant cytotoxic T cells and B cell epitopes in viral proteins. The purpose of this study was to analyze SARS CoV 2 in bats in Karst Malang Indonesia as vaccine candidates, diagnostic kits and immunotherapy.

Materials and Methods

Sample Collection

The research was conducted from October 2019 to September 2020 in a cave in the karst, Malang, Indonesia. The materials used in the study consisted of 70% alcohol and cotton which was used to anesthetize and preserve bat samples for specimen collection. The tools used to collect bat samples are mist nets, ropes, 2.5 - 3 m long poles to install fog nets, long-stemmed nets, calico bags, masks, scissors, long tweezers, identification books and cameras. Sampling of bat species was carried out by trapping method using Mist net[16]. Catching bats is carried out when the bats start their activities in the afternoon. The bats that are caught are then identified to determine their species, referring to Suyanto (2001). The bat is laid by tilting it (dorsal recumbency) over the styrofoam by fixing the proximal and distal wings using pins. Laparotomy surgery is performed ventral so that the abdominal cavity opens. The taken organs are separated and put into a container that already contains 10% PBF.

RNA extraction

RNA was extracted from bat organ samples with the Pure 96 MagNA System (Roche, Penzberg, Germany) and from cell culture supernatants with a mini RNA virus kit (QIAGEN, Hilden, Germany).

Real Time PCR

The 25 µL reaction contains 5 µL RNA, 12.5 µL 2×reaction buffer provided with Superscript III one-step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; contains 0.4 mM each of deoxyribont triphosphate (dNTP) and 3.2 mM magnesium sulfate), 1 µL reverse transcriptase / Taq mixture from the kit, 0.4 µL 50 mM magnesium sulfate solution (Invitrogen), and 1 µg nonacetylated bovine serum albumin. Thermal cycling was performed at 55 °C for 10 minutes for reverse transcription, followed by 95 °C for 3 minutes and then 45 cycles at 95 °C for 15 s, 58 °C for 30 s.

Sequencing

The amplification results used RT-PCR in the form of cDNA for sequencing. The sequencing reaction is carried out enzymatically, with dye terminator cycle sequencing. Before sequencing the PCR product must be purified to clean the PCR product from the rest of the

buffer and other reaction components so that it can get good sequencing results.

3-Dimensional Structure

SARS CoV 2 protein from Malang Karst bats in Indonesia using SwissProt.

Epitope predictive analysis

Prediction of B-cell epitopes from SARS CoV 2 protein in Malang Karst bats in Indonesia using IEDB. Next, we sent the predicted peptides to VaxiJen to determine whether the predicted epitopes could be prospective protective antigens that would build up the immune response.

Allergy prediction and non-toxic protection

In this study, extensive analysis of the predicted allergenicity of the predicted peptides was carried out using AllerTOP, then, we estimated the non-toxic protective antigens that perform ToxinPred.

Results and Discussion

Various efforts have been directed to the development of the COVID-19 vaccine, to prevent a pandemic and most of the developing vaccine candidates have used the SARS-CoV-2 protein.The immuno-informatics approach is also used for the identification of the SARS-CoV-2 vaccine candidate epitopes. It can be used to identify significant cytotoxic T cells and B cell epitopes in viral proteins (Gupta et al., 2006; Baruah and Bose, 2020). From the results of the research conducted, it was found that the three-dimensional structure of the SARS CoV-2 protein from the Karst Bats in Malang Indonesia.

The protection that currently available vaccines provide against viruses is based on neutralizing antibodies. Such antibodies usually block the interaction of the virus with viral cellular receptors or prevent the conformational changes required for viral fusion with membrane cells. The SARS-CoV-1 virus has been studied in great detail. Recent investigations have shown that the new SARS-CoV-2 virus uses the same strategy for entry into cells[17,18]. The interaction of S protein with ACE2 is well described for SARS-CoV-1 and -2 and relies on a specific domain in the protein, called the receptor binding domain (RBD). Indeed, most of the antibodies capable of neutralizing the corona virus are directed against RBD. Therefore, the main immune mechanism to avoid infection is through blocking of viral adherence to ACE2. Therefore, producing vaccine-inducing antibodies against RBD is the strategy used by most of the COVID-19 vaccine candidates. Recently it has been shown that RBD is glycosylated and methylated. In general, such post-translational modifications are difficult to reproduce in vaccines, meaning the vaccine may display a (slightly) different epitope from the virus. As a result, vaccine-induced antibodies are potentially cross-reactive and non-protective. Interestingly, however, the receptor interaction site (RIS) file that directly binds to ACE2 is not glycosylated, suggesting that this RIS may potentially be an ideal vaccine candidate. The second most common option is to use the entire S1 subunit. Other vaccine manufacturers use proteins and/or peptides that fuse with the cell membrane and therefore also have neutralizing epitopes. The SARS-CoV-2 fusion protein readily induces neutralizing antibodies in non-human primates. Proteins and peptides can be made more immunogenic by formulating them with powerful adjuvants. A potential challenge is that the induction of neutralizing antibodies is dependent on the display of the antigen in the correct conformation, that is, it is not guaranteed when the protein or peptide is expressed and displayed separately at the injection site[19].

The most obvious isotype to be induced by the COVID-19 vaccine is IgG, preferably the protective IgG1 and IgG3 subclasses. However, IgA may also be important for reducing infection of mucosal and epithelial cells in the respiratory tract, as well as endothelial cells, which may be very widely targeted by viruses. Although immunization of the mucosa on a large scale rapidly may be difficult, the use of adjuvants that stimulate IgA production may be an important consideration. TLR7/8 and TLR9 ligands are good candidates because they potentially promote the IgA response[20].

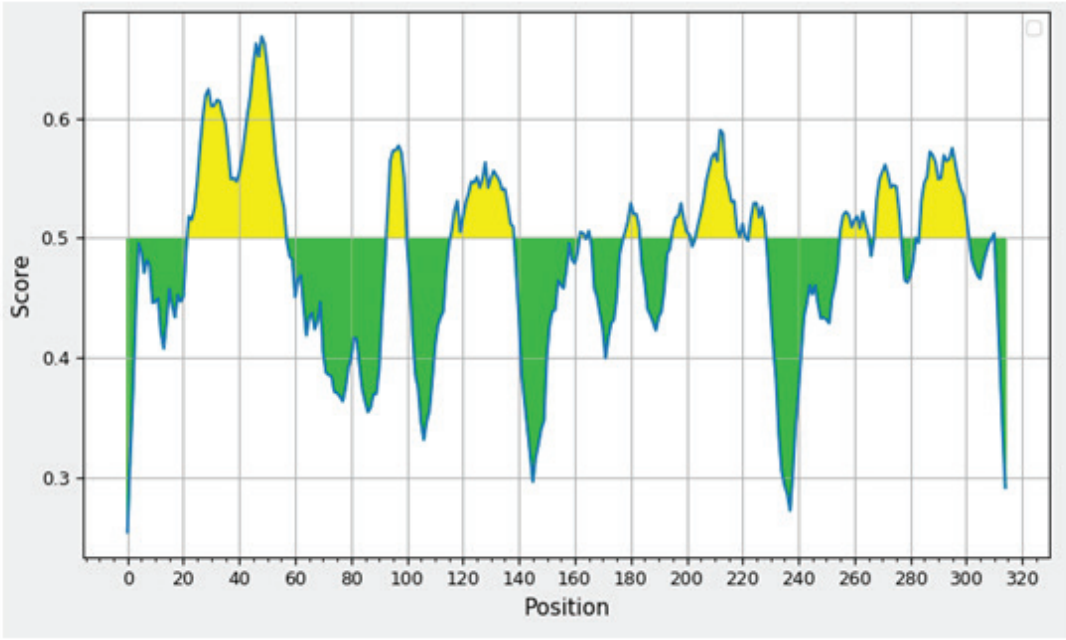

Figure 1. Prediction of Epitope Based on Cell B from SARS CoV 2 protein from Karst bats Malang Indonesia

Table 1. Prediction of protective antigens, allergens and toxins for SARS CoV 2 From Bats, Malang Karst

Peptides	Long	Position	Allergenicity prediction	Protective Antigens Prediction	Toxigenicity Prediction
IQRRNWPTHASKSPKRNYLLRGRNT SHRSVNRGSC	35	23-57	Probable Non-Allergen	Probable Antigen (0.6242)	Non-Toxin
IISTSRFCPGVTEREVFCENRTA	23	117-139	Probable Non-Allergen	Probable Antigen (0.8551)	Non-Toxin
VYTFPGNKPTNFRSLVDL	18	205-222	Probable Allergen	Probable non Antigen (0.1359)	Non-Toxin
VTRLSSAGCLR	11	256-266	Probable Allergen	Probable Antigen (0.5959)	Non-Toxin
KGKMESLVPGFNEKTHVQ	18	285-302	Probable Allergen	Probable Antigen (0.8644)	Non-Toxin

Conclusion

From the results of the research, it was found that the antigenic, non-allergen and non-toxic proteins of SARS CoV2 from Malang Karst bats were used as vaccines, immunotherapy and diagnostic kits.

Conflict of Interest : The authors declare that they have no conflict of interest.

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