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Development of prophylactic nucleic acid-based vaccine against SARS-CoV-2

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Summary

The newly emergent novel coronavirus nCoV-19, which was later named SARS-CoV-2, has been recently identified in Wuhan, China in 2019. As of 15th of February, a total of 73,335

cases had been reported globally with 1,873 reported deaths. Therefore, development of prophylactic vaccines against SARS-CoV-2 is a global priority. Currently, no licensed vaccines or therapeutics exist against SARS-CoV-2 or the other human

coronaviruses. Here, we propose to design, produce, and test prophylactic vaccine against SARS-CoV-2. As such, nucleic acid vaccine technology will be implemented

for rapid vaccine development. Successfully produced pDNA encoding various forms of engineered S genes will be tested in C57BL/6 mice for eliciting effective humoral antibody responses able to neutralize SARS-CoV-2 and coronaviruses' closely related strains. Upon successful completion, these SARS-CoV-2 vaccines can be further tested in clinical trails.

Registration details	
Status of the study	Accessible
Date of registration	2020-04-02
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1. General Information

Keywords

SARS-CoV-2, COVID-19, DNA Vaccine, Spike

Funding sources

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International code of classification

COVID-19

2. Study design

Introduction

Over the past two decades, three novel coronaviruses associated with severe human infections have been identified: SARS-CoV, MERS-CoV, and most recently SARS-CoV-2. These coronaviruses are associated with acute respiratory illness and can be life-threatening. While infections with coronaviruses are usually associated with mild illness in humans, SARS-CoV and MERS-CoV have been associated with severe illnesses with 10% and 40% mortality rates, respectively.

On December 2019, a novel human coronavirus that was later designated SARS-CoV-2 has emerged in Wuhan, China. As of February 15, more than 70,0000 cases have been reported across 28 countries with over 1000 reported deaths. So far, the number of deaths associated with infection with SARS-CoV-2 has been higher than in SARS-CoV or MERS-CoV. This re-emergence of SARS-CoV-2 with a strong propensity for human-to-human transmission has brought a global attention with high alert.

Viruses of the family Coronaviruses are single stranded, positive-sense, RNA viruses (Su et al., 2016). The coronaviruses are classified into four genera (α, β, γ, and δ) and their genome size ranges between 26,000 to 32,000 nucleotides in length (Su et al., 2016). Recent phylogenic evolutionary analyses revealed that COVID-19 belongs to betacoronaviruses genus, and share 88% identity with two bat derived SARS-like CoV (bat-SL-CoVZC45 and bat-SL-CoVZXC21) (Lu et al., 2020; York, 2020). The genome of COVID-19 shares about 79% similarity with SARS and about 50% similarity with MERS-CoV (Lu et al., 2020).

The enveloped coronaviruses contains three structural proteins; spike (S), envelope (E), and membrane (M). Typically, the S of coronaviruses is trimetric glycoprotein that belongs to class I fusion proteins (See et al., 2008). Each S monomer is cleaved into S1 and S2 domains; the S1 domain is responsible for virus binding to host cell receptor (Li et al., 2003; Wong et al, 2004), while S2 is responsible for fusion between the virus envelope and the host cell membrane (Czub et al., 2005; He et al., 2006). Similar to SARS-CoV-2, angiotensin-converting enzyme (ACE2) has been recently identified as the receptor of SARS-CoV-2 (Li et al., 2003; Wong et al., 2004; Zhou et al., 2020; Wu et al., 2020).

The role of S glycoprotein in receptor binding membrane fusion make it an attractive target for vaccine development for which neutralizing antibodies elicited against S protein after vaccination can inhibit virus binding or fusion to host cell membrane. Therefore, S glycoprotein is the major antigenic determinant in coronaviruses responsible for inducing effective neutralizing protective immune response against the virus.

Currently, no licensed vaccines or therapeutics against the SARS-CoV-2 exists. Given the urgency of this outbreak, a rapid development of prophylactic vaccines against the SARS-CoV-2 is a priority. Nucleic acid vaccines are perfect platform for rapid vaccine production; Bypassing the burdensome of conventional vaccine production, in pDNA vaccine, a gene of interest can be cloned into expression vector (pDNA) for a robust, generic, and highly scalable production (Li et al., 2016; Liu et al., 2016; Ahn et al., 2018; Ghaffarifar, 2018; Li and Erick, 2020). The levels of protective immune response elicited after DNA vaccine encoding S gene can be assessed. Upon successful completion, these SARS-CoV-2 vaccines can be tested in further human clinical trails.

Type of research

Exploratory

Hypothesis of your study

I propose to develop vaccine for the novel coronavirus SARS-CoV-2, based on nucleic acid vaccine technology. The vaccine is based on genetically optimized, remodeled spike (S) genes to elicit potent humoral antibody response. The S protein is known as the major antigenic determinant for the coronaviruses (CoV) family. The scope of immunogenic protection for the remodeled S immunogens ”vaccines” will be directed to closely related SARS-CoV-2 strains. Further, the incorporation of genetically modified S genes into plasmid DNA (pDNA) will provide excellent gene delivery and enhance in-vivo expression. Genetically modified S gene will be cloned, purified, and tested for the presence of antigenic epitopes. The immunogenicity and protective antibodies responses elicited by single or combined gene candidates will be evaluated. Most promising genetically engineered S vaccine candidate will be selected for further clinical studies (Phase I). Specific aims#1 Design, construct, and produce engineered pDNA plasmids displaying different constructs of S genes of SARS-CoV-2. • Optimization of gene constructs • Gene synthesis, cloning, and plasmid purification • Evaluate gene expression in-vitro Specific aim #2 Evaluate the immune response elicited in mice following I.M immunization with the candidate pDNA vaccines displaying engineered S genes inserts. • Immunize C57BL/6 mice through I.M with different S constructs to produce binding and neutralizing antibodies. • Assess the levels of binding and neutralizing antibodies elicited against S protein. Specific aim# 3 Mapping of antibody epitope elicited by pDNA vaccines constructs expressing S gene of SARS-CoV-2

Study design

TASK

Q1

Q2

Q3

Q4

Q1

Q2

Q3

Q4

Codon OPT

Gene synthesis

Cloning/Expression

pDNA production

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Animal study

Immunogenicity

Data analysis

Antibody mapping

Data analysis

Final report

Method of blinding

The investigator analyzing assessing the data will not aware of the experimental groups.

Method of randomization

Not applicable

3. Methods

3. 1. Design and synthesis of engineered pDNA expressing various forms of S genes constructs.

Description of the method

Optimization of genes constructs

The S gene segments will be optimized for mammalian codon usage (human) to enhance the transgene expression. In addition, a Kozak sequence will be also included.

Gene synthesis, cloning, and plasmid purification

Design and generate genetically modified S genes of SARS-CoV-2 expressing the following constructs: (Full length codon optimized S gene “S.opt.FL”, S gene with deletion of transmembrane region “S.opt.dTM”, S1 gene domain encoding the S1 domain “S1.opt”). Each of these constructs will be subjected to codon optimization for mammalian usage. Each of these constructs will be subcloned into an expression vector “pVax1 plasmid”. The pVax1 construct will be also modified to include addition of cytokines genes. Each gene constructs will include flanking region for Nhe I “upstream” and Bamh I “downstream” endonuclease restriction enzymes for a successful insertion of gene constructs into pVax1. These successfully cloned pDNA constructs representing individual engineered S will be further purified. DNA electrophoresis with restriction analysis will be further preformed to

verify the correct insertion of individual pDNA constructs with the correct sizes. Plasmids were produced from QIAGEN-plasmid MEGA and GIGA kits.

Evaluate gene expression in-vitro

Human embryonic kidney cells (HEK 293) cells will be used to express pDNA encoding S genes constructs in-vitro. To produce individual S proteins of SARS-CoV-2 displaying various forms of S proteins, these constructs will be transiently transfected with the cloned plasmid vector expressing individual synthetic S genes. Later, the produced S proteins will be harvested from cell supernatants and lysate. Each of produced S protein will be subjected to SDS-WB to confirm the expression of these proteins. MERS-CoV or SARS-CoV-2 antibodies will be used as a detection system.

Narcotic/analgesic treatment

Not applicable

Drugs/substances

Not applicable

Antibodies

Not applicable

Cell lines, viruses, DNA or RNA constructs and bacteria

Yes Synthetic DNA constructs expressing various forms of spike gene

3. 2. Evaluate the immune response elicited in mice following I.M immunization with the candidate pDNA vaccines displaying eng

Description of the method

Immunize C57BL/6 mice through I.M with different S constructs to produce binding and neutralizing antibodies.

Individual pDNA constructs encoding S.opt.FL and S1.opt that are successfully cloned and expressed, will be further evaluated for immunogenicity. Individual pDNA constructs will be administered in C57BL/6 mice, at 6-8 weeks of age, intramuscularly (I.M) at different pDNA doses (100 ug and 200 ug). Binding antibodies, neutralizing antibodies and cytokines measurements will be further assessed. Briefly 7 groups of 6 mice, 4-6 weeks old, will be immunized twice, at two weeks intervals by I.M immunization with pDNA S.opt.FL, pDNA S1.opt, a combination of two vaccines, DNA prime and protein boost, or PBS. Serum samples will be collected at week 0, 2, 4, 6, and 8.

Assess binding and neutralizing antibodies against S of SARS-CoV-2

Assessment of binding antibodies will be determined by ELISA measurement. ELISA. The level of serum S IgG antibodies elicited by each of pDNA SARS-CoV-2 constructs will be determined. A supernatant of a cloned and successfully expressed of S.opt.dtm will be directly coated to the plate. Further, measurement of neutralizing antibodies is important because virus neutralization is an indicator of immune protection from COVID-19. Mirconeutralization test will measure the serum level of neutralizing antibodies, for which pseudoviruses obtained through co-transfection of S.opt.FL with pN-L-3-4 vector will be used to assess the humoral protective response. Mice immunized with PBS will be the control group for immunization. Mice spleen will be isolated and used and fused with hybridoma cells for mAB screening screen and production.

Group

Mice per group

Vaccine

Dose per immunization

Route

1

6

S.opt.FL DNA + S.opt.FL DNA

100 ug

I.M

2

6

S.opt.FL DNA+ S.opt.FL protein

100 ug

I.M

3

6

S1.opt DNA+ S1.opt DNA

100 ug

I.M

4

6

S1.opt DNA + S1.opt protein

100 ug

I.M

5

6

S.opt.FL DNA +S.opt.FL protein

100 ug

I.M

6

6

S.opt.FL DNA+S1.opt Protein

100 ug

I.M

7

6

PBS

-

I.M

Narcotic/analgesic treatment

Not applicable

Drugs/substances

Not applicable

Antibodies

Not applicable

Cell lines, viruses, DNA or RNA constructs and bacteria

Not applicable

3. 3. Mapping of antibody epitope elicited by pDNA vaccines constructs expressing S gene of SARS-CoV-2

Description of the method

ASET array will be implemented to identify antigenic epitopes (at amino acids level) that stimulate the production of antibody responses in mice after immunization with pDNA expressing S genes of SARS-CoV-2. Sera at week 6 will be used to identify antibody epitopes using the high quality S and S1 antibodies produced after pDNA immunization.

Expected outcome

SARS-CoV-2 is the seventh human coronavirus that was recently discovered and was linked to the recent outbreak in Wuhan, China. It is urgently needed to develop effective vaccine to contain and prevent future SARS-CoV-2 outbreaks, globally. Nucleic acid vaccine technologies possess excellent features for the generation of vaccine against rapidly emerging and re-emerging viruses. As such, pDNA can selectively include a single gene of interest to induce rapid and robust immune response against a selected pathogen. Here, we propose to design and generate pDNA vaccine encoding S gene for the newly emerging human coronavirus (SARS-CoV-2). Upon immunization, pDNA carrying various gene constructs will be immunized in non-human models. Levels of binding antibodies and protective antibodies elicited in C57BL/6 mice will be evaluated. Furthermore, with the inherent properties of pDNA vaccines to elicit innate immune response, measurements of cytokines elicited after vaccination will be assessed. Isolated mABs can be valuable for future characterization of mAB that will provide important information on the antigenicity and immunogenicity of SARS-CoV-2.

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Narcotic/analgesic treatment

Not provided

Drugs/substances

Not provided

Antibodies

Not provided

Cell lines, viruses, DNA or RNA constructs and bacteria

Not provided

4. Statistics

4. 1. Student’s t-test

Assigned method(s)

Evaluate the immune response elicited in mice following I.M immunization with the candidate pDNA vaccines displaying eng

Main endpoints

Differences between immunized mice groups demonstrating S specific binding antibody responses as measured by ELISA and microneutralhzation antibody titers. A p value of less than 0.05 will be considered significant.

Secondary endpoints

Not provided

Sample size calculation

Immunization: 6 mice per group Control: PBS

Primary statistical analysis

Student’s t-test

Exclusion criteria

Non applicable

5. Animals

5. 1. Mice (Mus musculus)

Animal strain/breed

C57BL/6

Genetically modified

No

Sex

Female

Further characteristics of the animals (e.g. age, body weight, size)

4-6 weeks old.

Housing conditions

Research animal facility is available at the animal house facility at the institute for research and medical consultations. The facility is maintained by trained staff. The facility provide procurement facility, routine animal care, heath surveillance, and veterinary support.

Refinement

Not provided