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A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial

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ABSTRACT

Background: The severe acute respiratory syndrome (SARS) virus is a member of the *Coronaviridae* (CoV) family that first appeared in the Guangdong Province of China in 2002 and was recognized as an emerging infectious disease in March 2003. Over 8000 cases and 900 deaths occurred during the epidemic. We report the safety and immunogenicity of a SARS DNA vaccine in a Phase I human study.
Methods: A single-plasmid DNA vaccine encoding the Spike (S) glycoprotein was evaluated in 10 healthy adults. Nine subjects completed the 3 dose vaccination schedule and were evaluated for vaccine safety and immune responses. Immune response was assessed by intracellular cytokine staining (ICS), ELISpot, ELISA, and neutralization assays.
Results: The vaccine was well tolerated. SARS-CoV-specific antibody was detected by ELISA in 8 of 10 subjects and neutralizing antibody was detected in all subjects who received 3 doses of vaccine. SARS-CoV-specific CD4+ T-cell responses were detected in all vaccinees, and CD8+ T-cell responses in ~20% of individuals.
Conclusions: The VRC SARS DNA vaccine was well tolerated and produced cellular immune responses and neutralizing antibody in healthy adults.

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1. Background

The first cases of an atypical pneumonia appeared in Fosham, Guangdong Province, China in November 2002. By February 2003, over 700 cases of severe acute respiratory syndrome (SARS) were reported in the Guangdong Province of China. Before it was contained by public health isolation and quarantine measures, the epidemic spread to 25 countries over 5 continents, and affected 8422 people [1,2].

SARS infection causes respiratory disease and other organ systems, including the gastrointestinal tract, are also severely affected. The elderly and immunocompromised are more severely affected and suffer greater morbidity and mortality. By July 2003, 916 deaths had been attributed to the infection by this virus [2]. Based on serologic data from samples collected prior to the outbreak and retrospectively analyzed, up to 40% of individuals working in the animal trade were seropositive but had no history of illness [3], indicating that SARS may be either extremely mild or asymptomatic in some cases. Severe acute respiratory syndrome virus (SARS-CoV) is an enveloped RNA virus and a member of the *Coronaviridae* family that also includes other human pathogens which typically cause mild upper respiratory infections.

Coronaviruses are enveloped viruses with a positive-sense, single-stranded RNA genome. SARS-coronavirus (SARS-CoV) was unknown prior to the 2003 outbreak of disease and may be a mutant human coronavirus that acquired new virulence factors allowing for infection of the human population [4]. The genomic RNA is encased in nucleocapsid (N) protein, which is surrounded by a lipid membrane containing the Spike glycoprotein (S), membrane glycoprotein (M), and envelope (E) proteins. Oligomers of the S-glycoprotein form a characteristic spike that protrudes from the membrane [4,5]. Viral entry into host target cells appears to

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be mediated by SARS-CoV Spike (S) glycoprotein and is dependent on angiotensin-converting enzyme 2 (ACE2) as the functional receptor [6]. In addition to being responsible for attachment to the cellular receptor, S contains epitopes for viral neutralization and T-cell responses [7]. Studies performed by VRC investigators and colleagues have shown the importance of S-glycoprotein for coronavirus assembly and trafficking [8]. Other studies have demonstrated neutralization of pseudovirions expressing this protein by serum from convalescent SARS patients and the ability of the DNA plasmid vaccine described here to induce protective immunity by eliciting cellular and humoral immunity to SARS-CoV in animal models, including the generation of neutralizing antibodies (NAbs) measured in a plaque-reduction assay [8,9]. Studies performed in Beijing, China with serum from patients with SARS using a neutralization assay against a pseudotyped lentiviral vector bearing the S protein indicated that NAbs were first detected 5–10 days after onset of symptoms, peaked at 20–30 days and were sustained for more than 150 days [10].

The nature of the spread and the severity of illness prompted widespread attempts to identify and understand the disease. The cause of SARS was determined to be a novel coronavirus and the virus was fully sequenced by May 2003 [11,12]. Rapid identification and sequencing of the virus allowed scientists to begin developing candidate vaccines quickly. Currently, there are no licensed human SARS vaccines, and only one other vaccine clinical trial has been reported evaluating a whole-inactivated SARS vaccine candidate developed by Sinovac Biotech Co. Ltd. in China [13]. The current report describes the results of a candidate SARS DNA vaccine evaluated in a Phase I clinical trial in healthy adults initiated within 19 months after the sequence of the virus was initially published.

2. Methods

2.1. Study design

The VRC 301 protocol was a Phase I open-label study of the safety, tolerability, and immunogenicity of a SARS recombinant plasmid DNA vaccine encoding SARS Spike glycoprotein in healthy adult subjects. This single-site study was conducted at the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) in Bethesda, Maryland. Experimental guidelines of The U.S. Department of Health and Human Services were followed in the conduct of clinical research, and the protocol was approved by the NIAID Institutional Review Board. Ten subjects, ages 21–49, were enrolled in the study from December 13, 2004 through May 2, 2005. Three injections of vaccine, at a dose of 4 mg each, were administered, on study days 0, 28, and 56 at a 4 mg dose in the lateral deltoid muscle via the Biojector 2000® Needle-Free Injection Management System™. The dose and route studied in this trial were based on preclinical data and data from clinical trials of VRC DNA vaccines for other pathogens [14–16]. Subject safety was monitored by evaluating laboratory and clinical findings and adverse reactions at study visits and adverse events were coded with the Medical Dictionary for Regulatory Activities (MedDRA). Solicited symptoms of local and systemic reactogenicity, including pain, redness, swelling, myalgia, malaise, headache, chills, nausea and temperature, were collected by subject self report on 5-day diary cards following each vaccination. Subjects were followed for a total of 32 weeks and study visits were completed in December 2005.

2.2. Vaccine

The vaccine, VRC-SRSDNA015-00-VP, is composed of a single closed circular plasmid DNA macromolecule (VRC-8318) that has been produced in bacterial cell cultures containing a kanamycin selection medium. Bacterial cell growth is dependent upon the expression of the kanamycin resistance protein encoded by a portion of the plasmid DNA. Following growth of bacterial cells harboring the plasmid, the plasmid DNA is purified and the vaccine does not contain cellular or viral components. The plasmid encodes for a single protein cloned into the expression vector CMV/R, that has been previously described [17] and evaluated in clinical trials for candidate HIV and Ebola DNA vaccines [14,16]. The plasmid was constructed to produce a deletion mutant of the SARS Spike glycoprotein (Urbani strain, GenBank AY278741) with the cytoplasmic domain truncated (SΔCD) [9]. The VRC-SRSDNA015-00-VP vaccine is based upon cDNA expression of SARS Spike glycoprotein (Urbani strain) with codon-modification to optimize expression in human cells. It expresses the full sequence except for deletion of the last 13 COOH-terminal amino acids. Vaccine for the clinical trial was prepared under current Good Manufacturing Practice (cGMP) conditions by Vical, Inc. (La Jolla, CA). The vaccine met a lot of release specifications prior to administration. The DNA vaccine was manufactured at a 4 mg/ml concentration in phosphate-buffered saline (PBS).

2.3. Measurement of antibody responses by ELISA

VRC plasmid 8318 was expressed in 293 cells and purified for the major protein product. Duplicate wells of serial dilutions of the volunteer sera were incubated for 2 h at 37 °C on SARS spike antigen-coated/blocked plates, followed by biotin-conjugated antibody (60 min at room temperature), Streptavidine-horseradish peroxidase (30 min at room temperature) and tetramethylbenzidine (TMB) substrate (10 min at room temperature). Color development was stopped by addition of 0.9 M sulfuric acid and plates were read within 30 min at 450 nm on the Molecular Devices Spectramax 384-plus ELISA Plate reader (Sunnyvale, CA). Mean optical density (OD) for each dilution was corrected for the mean OD of the same dilution of the pre-immunization sample. Endpoint titers for each volunteer were established as the last dilution with a pre-immunization corrected OD >0.2.

2.4. Measurement of neutralizing antibody responses

Subject samples were assessed for the presence of vaccine-induced neutralizing antibody by two assays. First, in a luciferase reporter pseudotyped lentiviral-based assay serial dilutions of sera were incubated with HIV-1 based luciferase reporter virus particles expressing SARS Spike glycoprotein that were produced in 293 T cells as previously described [18]. Target cells were human renal adenocarcinoma cell line 786-O obtained from ATCC. The inhibitory concentration 80% (IC_{80}) is reported as a reciprocal dilution, and the analysis was performed using previously described methods [19]. Secondly, two-fold dilutions of heat-inactivated serum were assayed in quadruplicate wells of a 96-well plate using a starting dilution of 1:4. This microneutralization assay measured antibodies that neutralized the infectivity of 100 $TCID_{50}$ of SARS-CoV in Vero cell monolayers. The presence of viral cytopathic effect was read on days 3 and 4 and the dilution of serum that completely prevented cytopathic effect in 50% of the wells was calculated by the Reed–Muench formula [20].

2.5. Measurement of T-cell responses by ELISpot

ELISpot was performed on subject samples at baseline and at weeks 2, 6, 8, 10, 12, and 32 as previously described [16]. Cells were stimulated overnight with vaccine insert specific peptide pools at