Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
A recombinant baculovirus-expressed S glycoprotein vaccine elicits high titers of SARS-associated coronavirus (SARS-CoV) neutralizing antibodies in mice

Zhimin Zhou a,*, Penny Post a, Rick Chubet a, Katherine Holtz a, Clifton McPherson a, Martin Petric b, Manon Cox a

a Protein Sciences Corporation, 1000 Research Parkway, Meriden, CT 06450, USA
b BC Centre for Disease Control, 635W 12th Ave, Vancouver, BC, Canada V5Z 4B4

Received 22 November 2005; received in revised form 17 January 2006; accepted 24 January 2006
Available online 9 February 2006

Abstract

A recombinant SARS-CoV spike (S) glycoprotein vaccine produced in insect cells in a pre-clinical development stage is described. A truncated version of S glycoprotein, containing only the ecto-domain, as well as a His-tagged full-length version were cloned and expressed in a serum-free insect cell line, ExpresSF®. The proteins, purified to apparent homogeneity by liquid column chromatography, were formulated without adjuvant at 3, 9, 27, and 50 μg per dose in phosphate saline and used to immunize mice. Both antigens in each formulation elicited a strong immune response after two or three vaccinations with the antigen. Neutralizing antibody titers correlated closely with standard ELISA reactivity against the S glycoprotein. The truncated S protein was also formulated with an adjuvant, aluminum hydroxide, at 1 μg per dose (±adjuvant), and 5 μg per dose (±adjuvant). Significantly enhanced immune responses, manifested by higher titers of serum ELISA and viral neutralizing antibodies, were achieved in adjuvanted groups with fewer doses and lower concentration of S glycoprotein. These findings indicate that the ecto-domain of SARS-CoV S glycoprotein vaccine, with or without adjuvant, is immunogenic and induces high titers of virus neutralizing antibodies to levels similar to those achieved with the full S glycoprotein vaccine.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: SARS-CoV; Recombinant protein vaccine; Virus neutralization; Adjuvant; Aluminum hydroxide

1. Introduction

Severe acute respiratory syndrome (SARS) is a respiratory disease, whose main symptoms include fever, cough, shortness of breath, and pneumonia. The World Health Organization reported 8114 cases with 775 fatalities (WHO: Updated recommendations, October, 2004). The etiologic agent of SARS was shown to be a coronavirus, now designated as SARS-CoV [1–5]. The disease has the potential to re-appear as a new naturally acquired outbreak, by accidental- [6], or by intentional-release.

The development of SARS-CoV vaccines has become a priority for preparedness for future outbreaks to protect health care workers managing new cases as well as the general population. A Phase I clinical trial using killed whole SARS-CoV vaccine has been conducted by Sinovac Biotech Ltd. However, concerns have been raised for the use of inactivated virus vaccines based on observations with feline coronavirus. There are also several subunit vaccines in pre-clinical development, targeting SARS-CoV structural proteins, including the spike (S) and nucleocapsid (N) proteins. Other vaccine candidates include vector-based DNA vaccines [7–11], a combination of whole killed virus and DNA vaccines [12], inactivated whole virus vaccines [13,14], a combination of DNA and S-peptide vaccines [15], and other recombinant proteins and their fragments.
One of the most promising candidates for SARS-CoV protein vaccine is the S glycoprotein protein. Oligomers of this glycoprotein form large spikes in the viral envelope and mediate the binding of SARS-CoV to host cell through host cell receptor, the angiotensin converting enzyme II (ACE2) [16–19]. The receptor-binding domain (RBD) of the S glycoprotein has been mapped to residues 318–510 [20–22]. A complex structure of RBD and soluble ACE2 was determined at 2.9 Å [23]. The S protein and its fragments were shown to also induce neutralizing antibodies [16,24–33]. The S glycoprotein has been biochemically characterized [20,34], and its features were explored for therapeutic applications [20,35].

This report describes two pre-clinical studies in mice using purified subunit vaccines of SARS S glycoprotein consisting of the ecto-domain (transmembrane domain deleted) of this protein and a His-tagged full-length S glycoprotein, expressed in an insect cell system. Immunogenicity of the protein vaccines in an ELISA and in viral neutralizing antibody assays was determined together with the adjuvant effect of aluminum hydroxide.

2. Materials and methods

2.1. Recombinant protein vaccine

The gene encoding the S glycoprotein was cloned from a lysate of culture SARS-CoV 3200300841 (Passage #3) in Triozol LS Reagent (Sigma), which was kindly provided by Dr. Dean Erdman (CDC, Atlanta). Briefly, the cDNA was cloned in two steps due to the size of the gene (∼3.5 kb). The 5′ one-third of the ORF (Front), containing the N-terminus, was cloned into a baculovirus transfer vector pPSC12 (Protein Sciences Corporation, PSC), downstream from the baculovirus very late promoter of the polyhedrin gene. The insert was also in frame with the chitinase secretion signal sequence. The remainder of the gene was cloned into pUC18. Both inserts were sequenced (MWG Biotech) in full to confirm presence of entire gene. Two fragments of the ORF were then joined in pPSC12 using convenient restriction sites to assemble the complete SARS-CoV ORF and the insert and flanking regions of the vector were sequenced.

The regions encoding the transmembrane and cytoplasmic domains of SARS-CoV were deleted using site-directed PCR. This construct was named ΔTM S (Fig. 1A). To assist purification of the full-length S protein, a His6-tag was constructed to the C-terminus of the protein (Fig. 1A). The His6-tagged full-length S glycoprotein was expressed in and secreted from the recombinant baculovirus-infected cells, while the His-tagged full-length S glycoprotein was not secreted.

Both ΔTM S and His-tagged full-length S glycoproteins were purified to apparent homogeneity by column chromatography (Fig. 1B and C) before formulation. Protein identity was determined by N-terminal peptide sequencing and Western blotting with anti-His6-tag monoclonal antibody (QIAGEN), and/or anti-SARS S sera (PSC). Purity of His-tagged full-length S (>90%) and ΔTM S (>95%) was determined by densitometry (Quantity One Densitometry).
Table 1
Mouse immunogenicity study without adjuvant

| Group | Test article | Dose (µg) | # Mice/day of blood sampling |
|-------|--------------|-----------|-----------------------------|
| 1     | ΔTM S        | 3         | Day 1<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 2     | ΔTM S        | 9         | Day 1 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 3     | FL His-tag   | 9         | Day 1 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 4     | ΔTM S        | 27        | Day 1 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 5     | ΔTM S        | 50        | Day 1 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 6     | Vehicle      | –         | Day 0<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |

<sup>a</sup> Dose day. Blood was collected prior to the administration of test article. For example, on Day 15, eight mice, half male and half female, of the total 40 mice in Group 1 that received one dose (50 µg/ΔTM S) were bled. The rest of 32 mice were immunized with the second dose of 3 µg/ΔTM S.

Software, BioRad. Binding of S proteins to ACE2 (R&D Systems Inc.) was tested in a standard ELISA. In this assay, ACE2 served as a capture molecule and an anti-SARS S monoclonal antibody or anti-sera was used as a detection reagent. There was a linear relationship between the optical density and SARS S protein concentration in a range of 0.1–1.0 µg/ml. A second manuscript detailed in cloning, expression, purification, and characterization of the S proteins is in preparation.

2.2. Formulation

In the preparation of the vaccine, the ΔTM S protein was formulated in 20 mM sodium phosphate saline, pH 7.0, at four dose levels, 3, 9, 27, and 50 µg/50 µl, whereas the His-tagged full-length S glycoprotein was formulated at 9 µg/50 µl. The PBS used in the formulations served as a vehicle control (Table 1). In the follow-up study of vaccine with adjuvant (Alhydrogel<sup>®</sup>, Accurate Chemical and Scientific Corporation, Westbury, NY), ΔTM S protein was formulated in 2.5 mM sodium phosphate saline, pH 7.0, containing 1.6 mg aluminum/ml. In these preparations, ΔTM S was formulated at 1 µg/50 µl (± aluminum), 5 µg/50 µl (± aluminum), 50 µg/50 µl (− aluminum) and the His-tagged full-length S glycoprotein was formulated at 5 µg/50 µl. Alhydrogel in 2.5 mM PBS and PBS served as control (Table 2). Non-adjuvant vaccine preparations were stored at −20 °C, while adjuvanted vaccine preparations were stored at 2–8 °C.

Table 2
Mouse immunogenicity study with adjuvant aluminum hydroxide

| Group | Test article | Dose (µg) | # Mice/day of blood sampling |
|-------|--------------|-----------|-----------------------------|
| 1     | ΔTM S        | 1         | Day 0<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 2     | ΔTM S + alum | 1         | Day 0<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 3     | ΔTM S        | 5         | Day 0<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 4     | ΔTM S + alum | 5         | Day 0<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 5     | FL His-tag   | 5         | Day 0<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 6     | ΔTM S        | 50        | Day 0<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 7     | Vehicle      | –         | Day 0<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |
| 8     | Alhydrogel   | –         | Day 0<sup>a</sup> 8Day 15<sup>a</sup> 8Day 30<sup>a</sup> 8Day 45<sup>a</sup> 8Day 60 8Day 75 8 |

<sup>a</sup> Dose day.

2.3. Immunization

Both non-adjuvanted and adjuvanted recombinant S glycoproteins were tested in mice for immunogenicity, by IIT Research Institute (ITRI). The vaccine preparations were administered to male and female CD1 mice (VAF/Plus, Charles River Laboratories, Kingston, NY) by intramuscular (IM) injection according to the study designs shown in Tables 1 and 2. The animals were weighed and assigned to treatment groups using a constrained random process such that all groups tested were comparable in pre-test body weight, and the weight variation of each animal used would not exceed ±20% of the mean weight. The animals received 50 µl of the dose formulation containing graded doses of the two antigen preparations. On the days specified, animals were euthanized and sera collected.

2.4. Anti-SARS ΔTM S end point titers

Anti-ΔTM S titer of each serum was determined by ELISA. Briefly, a 96-well plate (NUNC-ImmuNo Maxi-Sorp) was coated with 1 µg/ml of purified ΔTM S protein in PBS and incubated overnight at 2–8 °C. Serial two-fold dilutions of mouse sera were stored at −20 °C, while adjuvanted vaccine preparations were stored at 2–8 °C.
coated with ΔTM S protein. The plate was incubated at room temperature for 1 h. After washing with PBS, goat anti-mouse IgG peroxidase conjugate (Amersham-Pharmacia Biotech), diluted 1:2000 with the antibody buffer, was added to the wells of the plate. After a further 1 h incubation at room temperature, the plate was washed with PBS and a preparation of 3.3’-5.5’-tetramethylbenzidine (TMB, liquid substrate system for ELISA, Sigma) was added as a peroxidase substrate. The plate was incubated at 37 °C for 30 min, the color stabilized by adding 100 μl of 3N H2SO4 to each well, whose OD 450 was then monitored on a Multiskan EX plate reader.

Sera were tested in singlet, and both positive and negative controls, pooled from eight mice, respectively, were also included in each ELISA plate. Geometric mean titer (GMT) of each dose at each bleed day was calculated based on titers of eight mice.

2.5. SARS-CoV-neutralization test

The virus neutralization (VN) test as described by [12] was performed. Vero-E6 cells were grown in 96-well cell culture plate until confluent monolayers formed. All sera were heated to 56 °C for 30 min before testing and assayed in duplicate. Sera were subjected to two-fold serial dilutions from 1:10 to 1: 5120 in a microtiter plate and 100 μl of SARS-CoV strain Tor 2, P4 added to each well and the preparation incubated at 37 °C for 2 h, a human SARS-CoV convalescent serum and a non-immune human serum were used as positive and negative controls. After incubation, 100 μl of serum/virus mixture was transferred from each well of the incubation plate to a 96-well cell culture plate containing Vero-E6 cells and the plate incubated at 37 °C for 3 days. The plates were then evaluated for the presence of characteristic SARS-CoV cytopathic effect (CPE) [12]. A neutralization titer was defined as the reciprocal of the serum dilution in the well immediately before the well manifesting CPE. A GMT was calculated for each dose for each bleed day, based on the titers obtained from eight mice.

3. Results

3.1. Immunogenicity of ΔTM S vaccine without adjuvant

The first study was designed to test immunogenicity of the recombinant SARS-CoV ΔTM S glycoprotein vaccine in mice. Ecto-domain of SARS-CoV glycoprotein, ΔTM S, was produced using a baculovirus-insect cell expression system in a serum-free cell line, ExpressSF®, and purified to apparent homogeneity (manuscript in preparation). This protein was shown to bind to soluble ACE2 [18,19] (R&D Systems Inc.) in an ELISA assay (Zhou, unpublished result), demonstrating that it has the conformation required for functional binding to the receptor. The protein was formulated in 20 mM sodium phosphate buffer, pH 7.0, containing 150 mM NaCl at dose levels of 3, 9, 27, and 50 μg/50 μl preparation of His-tagged full-length SARS S glycoprotein was included in the assessment to provide an insight into differences in immunogenicity between the truncated and full-length proteins.

Groups of eight mice, equally male and female, were injected intramuscularly (IM) for a total of one, two, three or four doses every 15 days (study days 1, 15, 30, and 45) (Table 1). No animals died during the study and no test material-related adverse clinical signs were noted. All the animals gained weight, and there were no significant differences in body weight or body weight gain between the groups. Sera were collected from all animals and tested for response to SARS-CoV by ELISA. A GMT was calculated based on serum titers of each group at each bleed day. GMTs of each vaccine group were calculated based on serum titers of each group at each bleed day. GMTs of each vaccine group were calculated based on serum titers of each group at each bleed day.

Groups of eight mice, equally male and female, were injected intramuscularly (IM) for a total of one, two, three or four doses every 15 days (study days 1, 15, 30, and 45) (Table 1). No animals died during the study and no test material-related adverse clinical signs were noted. All the animals gained weight, and there were no significant differences in body weight or body weight gain between the groups. Sera were collected from all animals and tested for response to SARS-CoV by ELISA. A GMT was calculated based on serum titers of each group at each bleed day. GMTs of each vaccine group were calculated based on serum titers of each group at each bleed day. GMTs of each vaccine group were calculated based on serum titers of each group at each bleed day.

Table 3

| Group | Test article | Dose (μg) | GMT Day 1 | GMT Day 15 | GMT Day 30 | GMT Day 45 | GMT Day 60 | GMT Day 75 |
|-------|-------------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 1     | ΔTM S       | 3        | 6666      | 43009     | 152219    | 98701     | 90150     |
| 2     | ΔTM S       | 9        | 6727      | 45255     | 165995    | 98701     | 128000    |
| 3     | FL His-tag  | 9        | 3175      | 19027     | 152219    | 279442    | 215689    |
| 4     | ΔTM S       | 27       | 5187      | 98701     | 152219    | 279442    | 215689    |
| 5     | ΔTM S       | 50       | 9514      | 139585    | 304437    | 234753    | 234753    |
| 6     | Vehicle     | <100      | <100      | <100      | <100      | <100      | <100      |
Table 4

| Group | Test article | Dose (µg) | GMT   |
|-------|--------------|-----------|-------|
| 1     | ΔTM S        | 3         | Day 1 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 |
| 2     | ΔTM S        | 9         | –     | 20     | 59     | 174    | 87     | 80     |
| 3     | FL His-tag   | 9         | –     | 14     | 67     | 72     | 87     | 135    |
| 4     | ΔTM S        | 27        | –     | 10     | 87     | 98     | 226    | 207    |
| 5     | ΔTM S        | 50        | –     | 13     | 190    | 207    | 207    | 174    |
| 6     | Vehicle      | –         | <10   | <10    | <10    | <10    | <10    | <10    |

in Table 4, the GMT peaked after four injections except for 3 µg dose group, which was highest after three injections. There is a good correlation between antibodies measured by ELISA and VN antibodies (Tables 3 and 4), suggesting that the ELISA may be used as a "surrogate" to measure functional antibodies if SARS-CoV-neutralization test is not available.

3.2. Immunogenicity using aluminum hydroxide as adjuvant

To evaluate whether the inclusion of an adjuvant into the SARS-CoV preparations would alter the ELISA and VN titers, a second mouse immunogenicity study was performed. Two preparations of ΔTM S at 1 and 5 µg/50 µl, were formulated with or without adjuvant, Al(OH)₃. Each adjuvanted dose (50 µl) contained 80 µg of aluminum, or 1.6 mg/ml, equivalent to 800 µg of aluminum in a 0.5 ml human dose. Due to competition of divalent cation phosphate for Al(OH)₃ binding, phosphate concentration of the formulation buffer was reduced from 20 mM (first study) to 2.5 mM. At this concentration, at least 95% of ΔTM S in 5 µg dose (5 µg/50 µl or 100 µg/ml) was adsorbed to aluminum hydroxide (80 µg/50 µl or 1.6 mg/ml) (data not shown). AlPO₄ ("ADJU-PHOS," Accurate Chemical and Scientific Corporation, Westbury, NY), was also evaluated for ΔTM S absorption and was found to be less efficient for aluminum hydroxide binding than Al(OH)₃ (data not shown).

Two non-adjuvanted preparations containing, 5 µg/50 µl of His-tagged full-length SARS S glycoprotein and 50 µg/50 µl of ΔTM S, were included to compare performance of non-adjuvanted preparations to those containing adjuvant.

Groups of eight mice, equally male and female, were injected intramuscularly (IM) for a total of one, two, three or four doses every 15 days (study days 0, 15, 30, and 45) (Table 2). In this study, one animal died and another suffered from weight loss. Both of the animals were in the high dose group (50 µg/50 µl). No other test material-related adverse clinical signs were noted. There were no significant differences in body weight or body weight gain between the groups. Additionally, no significant differences were observed in body temperatures 24 h following dosing. Therefore, it was determined that the described events were incidental and consistent with normal variations observed in mice and not biologically significant.

Sera from all the animals were collected. End point titers of the sera were determined by ELISA. GMTs were calculated for each group and the results are summarized in Table 5.

GMTs of all six dose groups peaked on Day 60 after four injections, and dose responses were clearly demonstrated (Fig. 2B). As observed in the first study, a limited immune response was observed across all the groups after
primary immunization, i.e., a single dose does not appear to be adequate with or without the use of an adjuvant. Titers of mice receiving PBS as a control remained at baseline throughout the study. The adjuvanting effect of Al(OH)3 was evident across all remaining time points following the second injection. Administration of adjuvanted vaccines resulted in GMTs several fold higher compared to corresponding non-adjuvanted vaccines. For instance, adjuvanted 1 µg group had titers similar to or higher than the non-adjuvant group.

Table 5: ELISA GMTs of mouse anti-ΔTM S sera collected from the second mouse immunogenicity study with adjuvant aluminum hydroxide

| Group           | Test article | Dose (µg) | GMT   |
|-----------------|--------------|----------|-------|
|                 |              |          | Day 0 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 |
| 1               | ΔTM S        | 1        | –     | 2000   | 4000   | 340/6 | 82998 | 38015 |
| 2               | ΔTM S        | 1 + alum | –     | 9752   | 124800 | 215269| 459559 | 152219 |
| 3               | ΔTM S        | 5        | –     | 2828   | 6400   | 58688 | 215269 | 76109 |
| 4               | ΔTM S        | 5 + alum | –     | 8833   | 394606 | 394406| 789612 | 362019 |
| 5               | FL His-tag   | 5        | –     | 2757   | 107615 | 215269| 254753 | 197465 |
| 6               | ΔTM S        | 50       | –     | 7127   | 17500  | 256000| 558349 | 115933 |
| 7               | Alhydrogel   | –        | –     | <100   | <100   | <100  | <100  | <100  |
| 8               | Vehicle      | –        | –     | <100   | <100   | <100  | <100  | <100  |

Table 6: GMTs of virus neutralization test of mouse anti-ΔTM S sera collected from the second mouse immunogenicity study with adjuvant

| Group           | Test article | Dose (µg) | GMT   |
|-----------------|--------------|----------|-------|
|                 |              |          | Day 0 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 |
| 1               | ΔTM S        | 1        | –     | 20     | 36     | 59    | 177   | 104   |
| 2               | ΔTM S        | 1 + alum | –     | 20     | 238    | 390   | 761   | 381   |
| 3               | ΔTM S        | 5        | –     | 10     | 66     | 101   | 538   | 269   |
| 4               | ΔTM S        | 5 + alum | –     | 25     | 226    | 698   | 1396  | 1174  |
| 5               | FL His-tag   | 5        | –     | 10     | 67     | 195   | 269   | 587   |
| 6               | ΔTM S        | 50       | –     | 10     | 67     | 195   | 269   | 587   |
| 7               | Alhydrogel   | –        | –     | <10    | <10    | <10   | <10   | <10   |
| 8               | Vehicle      | –        | –     | <10    | <10    | <10   | <10   | <10   |

primary immunization, i.e., a single dose does not appear to be adequate with or without the use of an adjuvant. Titers of mice receiving PBS as a control remained at baseline throughout the study. The adjuvanting effect of Al(OH)3 was evident across all remaining time points following the second injection. Administration of adjuvanted vaccines resulted in GMTs several fold higher compared to corresponding non-adjuvanted vaccines. For instance, adjuvanted 1 µg group had titers similar to or higher than the non-adjuvant group. The highest GMT of this dose group was 1:1396 at Day 60.

Overall, the recombinant protein vaccine ΔTM S was shown to be immunogenic in mice and to elicit SARS-CoV-neutralizing antibodies. This vaccine shows an increasing antibody response with increasing doses and antigen concentration. ΔTM S appears to perform equivalent to His-tagged full-length S glycoprotein in both studies. Adjuvanticity of aluminum hydroxide was clearly demonstrated at two different dose levels, 1 and 5 µg.

4. Discussion

A number of studies have shown that S protein and its fragments induce SARS-CoV-neutralizing antibodies [16,24–33]. The receptor-binding domain (RBD) in the S1 region plays a critical role in the neutralizing antibody induction as well as ACE2 binding and viral entry. Jiang and coworkers showed that RBD-Fc fusion induced high neutralizing antibody titers in mice, with a mean 50% titer of 1/15,360 against SARS-CoV infection [29]. Depletion of RBD-specific antibodies from sera significantly reduced serum-neutralizing capability [28], indicating this domain is dominant for the neutralizing antibody induction.

In this report both SARS S subunit vaccines, His-tagged full-length S and ΔTM S, contain RBD. With or without adjuvant, they were shown to be effective antigens in eliciting a strong neutralizing antibody response in mice. This is encouraging since highly purified proteins by themselves may be poorly immunogenic or non-immunogenic
The question was raised whether an adjuvant could further enhance immunogenicity. Aluminum hydroxide (Alum), introduced in the 1920’s, has been frequently used as an adjuvant. It enhances immunogenicity by converting soluble protein vaccines into particulate mass to make them more suitable for ingestion by antigen-presenting cells such as macrophages [36]. The adjuvant potential of aluminum hydroxide was explored in the second mouse immunogenicity study. As shown in Fig. 3B, adjuvant effect was obvious in both 1 and 5 µg ∆TM S + Alum groups on Day 30 after the second administration. There were several fold differences in titer between adjuvanted and non-adjuvanted groups. Similar observations were obtained in VN GMT. The 1 µg adjuvanted dose induced an immune response equivalent to that of 50 µg non-adjuvanted dose, suggesting that formulation of ∆TM S with aluminum hydroxide results in a dramatic improvement in immune response. It was however observed that even with the inclusion of adjuvant, multiple doses were required to elicit a strong antibody response.

There appears to be a strong correlation between GMTs obtained with ELISA and those determined in the actual VN assay, suggesting that the ELISA may be used as a “surrogate” assay for the actual VN assay while investigating potential antigens and their immune responses. This is an important finding since the VN assay, which uses the live virus must to be performed under BSL-3 conditions and hence could be reserved for the final assessment.

The biological significance of the higher GMT observed while adjuvating the antigen with Alum remains to be determined. We are planning to conduct a SARS-CoV challenging study in ferrets to study whether adjuvanting of the vaccine can indeed result in better protection against disease. Proof that the vaccine is effective in preventing disease in ferrets will provide valuable evidence to plan a Phase I clinical study to evaluate the immunogenicity of this recombinant protein vaccine in human subjects.

Acknowledgements

This study was sponsored by NIH/NIAID/DMID grant N01-AI-30023. Authors thank NIH/NIAID colleagues, Lydia Fulk, Fred Cassels, Sonnie Kim, Sue Yuan, Nicholas Obiri, and Michael Kennedy for their supports on the project. We acknowledge Tomas Kuchar, Kathryne Rizzo, Jennifer Knowlton, Jennifer Lynes, Tina Henry, Kelly Cacchillo, and David Lawrence for technical assistance. We also wish to thank William Mega (IITRI) for the animal work described in this paper and Wafaa Mahmoud for reviewing the manuscript.

References

[1] Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003;348:1953-66.
Z. Zhou et al. / Vaccine 24 (2006) 3624–3631

[2] Drosten C, Gunther S, Preiser W, van der Werf S, Brodh RR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. Nat Med 2003;9:1319–23.

[3] Peiris JS, Lau ST, Poon LL, Guan Y, Yam LY, Lim W, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003;361:1319–25.

[4] Marra MA, Jones SA, Astell CR, Holz RA, Brooks-Wilson A, Butterfield YS, et al. Granome sequence of the SARS-associated coronavirus. Science 2003;300:1399–404.

[5] Bota PA, Oborne ME, Monsen ST, Nis WA, Campagnoli R, Lesnigil JP, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 2003;300:1394–9.

[6] Liang G, Chen Q, Xu J, Liu Y, Lim W, Peiris JS, et al. Laboratory diagnosis of four recent sporadic cases of community-acquired SARS. Guangdong Province, China. Chin Emerg Infect Dis 2004;10:1774–81.

[7] Yang ZY, Kong WP, Huang Y, Roberts A, Murphy BR, Subbarao K, et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature 2004;428:561–4.

[8] He H, Yang Y, Qin X, Xu W, Wang Y, Liu X, et al. Construction of a subunit expression plasmidencoding partial S gene fragments of the SARS-CoV and its potential utility as a DNA vaccine. DNA Cell Biol 2005;24:516–20.

[9] Okada M, Takemoto Y, Okuno Y, Hashimoto S, Yoshida S, Fukunaga Y, et al. Construction of vaccines against SARS coronavirus in mice and SCID-PBL-hu mice. Vaccine 2005;23:2289–72.

[10] Wang S, Chou TH, Sikluksky PV, Huang S, Lawrence JM, Cao H, et al. Identification of two neutralizing regions on the severe acute respiratory syndrome coronavirus spike glycoprotein produced from the mammalian expression system. J Virol 2005;79:1906–10.

[11] Bisht H, Roberts A, Vogel L, Bukreyev A, Collins P, Murphy BR, et al. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protects immunized mice. Proc Natl Acad Sci USA 2004;101:6641–6.

[12] Zakharichuk AN, Liu Q, Petri M, Balbok LA. Augmentation of immune responses to SARS coronavirus by a combination of DNA and whole killed virus vaccines. Vaccine 2005;23:4385–91.

[13] He Y, Zhou Y, Siddiqua P, Jiang S. Inactivated SARS-CoV vaccine elicits high titers of spike protein-specific antibodies that block receptor binding and virus entry. Biochem Biophys Res Commun 2004;324:773–81.

[14] Bischof U, Bukreyev A, Yang L, Lamardane EW, Murphy BR, Subbarao K, et al. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proc Natl Acad Sci USA 2004;101:9804–9.

[15] He Y, Zhou Q, Liu S, Zhou Y, Yang B, Li J, et al. Identification of an immunodominant site on the spike protein of severe acute respiratory syndrome coronavirus: implications for designing SARS vaccines. Virology 2005;334:74–82.

[16] He Y, Lu H, Siddiqua P, Zhou Y, Jiang S. Receptor-binding domain of severe acute respiratory syndrome coronavirus receptor for cellular entry. Proc Natl Acad Sci USA 2004;101:959–64.

[17] Babcock GJ, Esshaki DJ, Thomas Jr WD, Ambrosino DM. Angiotensin-converting enzyme 2 is a functional receptor for SARS coronavirus. Nature 2003;426:450–4.

[18] Virology 2004;334:445–52.

[19] Peiris JS, Guan Y, Yuen KY. Severe acute respiratory syndrome. Nat Med 2004;10:508–97.

[20] He Y, Zhou W, Hu H, Luo B, Chen J, Li W, et al. Identification of immunodominant sites on the spike protein of severe acute respiratory syndrome coronavirus: implications for developing SARS diagnostics and vaccines. J Immunol 2004;173:4050–7.

[21] He Y, Zhou W, Liu S, Kou Z, Li W, Furaz M, et al. Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implications for developing subunit vaccine. Biochem Biophys Res Commun 2004;324:773–81.

[22] Marra MA, Jones SA, Astell CR, Holt RA, Brooks-Wilson A, Butcher ME, et al. Identification of the SARS coronavirus spike glycoprotein in rats. Virus Res 2005;112:24–31.

[23] Lai MM, Cavanagh D. The molecular biology of coronaviruses. Adv Virus Res 1997;49:1–100.

[24] Li W, Moore MJ, Choe H, Farzan M. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 2004;279:3197–201.

[25] Faber M, Laminarde EW, Roberts A, Ricci AB, Koprowski H, Dietzschold B, et al. A single immunization with a diabody-based vector expressing severe acute respiratory syndrome coronavirus (SARS-CoV) S protein results in the production of high levels of SARS-CoV-neutralizing antibodies. J Gen Virol 2005;86:1435–40.

[26] Liu YR, Wu LZ, Huang BO, Huang JL, Zhang YL, Ke ML, et al. Adenoviral expression of a truncated S1 subunit of SARS-CoV spike protein results in specific humoral immune responses against SARS-CoV in rats. Virol Sin 2005;11:22–31.

[27] Chen Z, Zhang L, Qin C, Bu L, Yi CE, Zhang F, et al. Reconstituent modified vaccinia virus Ankara expressing the spike glycoprotein of severe acute respiratory syndrome coronavirus induces protective neutralizing antibodies primarily targeting the receptor binding region. J Virol 2005;79:2676–80.

[28] Bischof H, Roberts A, Vogel L, Subbarao K, Moss B. Neutralizing antibody and protective immunity to SARS coronavirus infection of mice induced by a soluble recombinant polypeptide containing an N-terminal segment of the spike glycoprotein. Virology 2005;334:160–5.

[29] Xiao X, Dimitrov DS. The SARS-CoV S glycoprotein: expression and functional characterization. Biochem Biophys Res Commun 2003;312:1159–64.

[30] Wong SK, Li W, Moore MD, Choe H, Furaz M, A. 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 2004;279:3197–201.

[31] Barouch DH, Esfiaki DJ, Thomas Jr WD, Ambrosino DM. Amino acids 270 to 510 of the severe acute respiratory syndrome coronavirus spike protein are required for interaction with receptor. J Virol 2004;78:10328–35.

[32] Janeway CA, Travers P, Walport M, Shlomchik M. Immunobiology. 5th ed. New York: Garland Publishing; 2001.