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.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 319 (2004) 929–935
BBRC
www.elsevier.com/locate/ybbrc

Identification of two antigenic epitopes on SARS-CoV spike protein

Ronghong Hua, Yanjun Zhou, Yunfeng Wang, Yuzhuo Hua, and Guangzhi Tong*

National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, PR China

Received 8 April 2004
Available online 28 May 2004

Abstract

The spike (S) protein of severe acute respiratory syndrome-coronavirus (SARS-CoV) is a major virion structural protein. It plays an important role in interaction with receptor and inducing neutralizing antibodies. In the study, six tentative antigenic epitopes (S1 S2 S3 S4 S5 S6) of the spike protein of SARS-CoV were predicted by bio-informatics analysis, and a multi-epitope chimeric gene of S1–S2–S3–S4–S5–S6 was synthesized and fused to downstream GST gene in pGEX-6p-1. The Western blotting demonstrated that SARS patient convalescent serum could recognize the recombinant fusion protein. A number of monoclonal antibodies were developed against the fusion protein. In further, the six predicted epitope genes were individually fused to GST of pGEX-6p-1 and expressed in *Escherichia coli* BL21, respectively. Among six fusion peptides, S5 reacted with monoclonal antibody D3C5 and S2 reacted with monoclonal antibody D3D1 against spike protein of SARS-CoV. The epitopes recognized by monoclonal antibodies D3C5 and D3D1 are linear, and correspond to 447–458 and 789–799 amino acids of spike protein of SARS-CoV, respectively. Identification of antigenic epitope of spike protein of SARS-CoV could provide the basis for the development of immunity-based prophylactic, therapeutic, and diagnostic techniques for the control of severe acute respiratory syndrome.

Keywords: Severe acute respiratory syndrome; Spike protein; Epitope; Monoclonal antibody

Severe acute respiratory syndrome (SARS) first appeared in Guangdong province, China, in Nov. 2002. Then this disease spread to several other countries around the world quickly and caused hundreds of deaths. Based on the work of world SARS research net, WHO declared a novel coronavirus is the pathogen of SARS on 16th April 2003, and referred to it as severe acute respiratory syndrome-coronavirus (SARS-CoV) [1–3].

SARS-CoV is enveloped, positive-sense, ssRNA virus. The genome of SARS-CoV is about 29.7 kb in length, with 11 open reading frames, and the genomic organization is similar to those of other coronaviruses [4]. The gene sequence and amino acid sequence have very low homology with any other known animal coronaviruses [4–6]. Though recently SARS-CoV-like viruses were isolated from Himalayan palm civets [7], these viruses have not been found in any animal or human being before 2003. And the phylogenetic analysis indicated that the new virus is not related to the known group 1, 2, and 3 coronaviruses and represents a novel coronavirus [8]. So it is proposed as representing a fourth group within the genus *Coronavirus*, the group 4 coronavirus. Based on available information of other coronaviruses, the spike glycoprotein is the main virulence factor of coronavirus [9]. Spike protein has multiple functions that are involved in specific receptor binding, cell membrane fusion, and protease susceptibility and activation. Spike protein has good antigenicity and could induce neutralization antibodies. These features make it be a suitable candidate for genetic engineering subunit vaccine and diagnostic applications.

In order to develop some basis for prophylactic and diagnostic use, it is important to map the epitopes of spike protein. In the study, two antigenic epitopes were identified by monoclonal antibodies.

* Corresponding author. Fax: +86-451-82734181.
E-mail address: gztong@public.hr.hl.cn (G. Tong).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.05.066

Materials and methods

Prediction of epitopes on spike protein. Spike protein sequence is deduced from SARS-CoV genome of strain BJ01 (GenBank Accession No. AY278488). Computerized algorithms were used to predict the hydrophilicity [10], surface probability [11], antigenic index [12], and secondary structure [13]. These analyses were performed with the biocomputing software program Laser gene-DNASTAR. According to the hydrophilicity, surface probability, antigenic index, and secondary structure, six segments (designated as S1, S2, S3, S4, S5, and S6) that possibly contain B-cell epitope were selected (Fig. 1).

Construction of a multi-epitope gene. A chimeric gene containing S1–S6 was constructed by lining up the six segments. The sequence of chimeric gene is as follows: ct**ggatcc**aatactaggaacattgatgctacttcaactgg taattataat*ggaccgggga*aagcttaggccctttgagagagacatatctaatgt*gggaccgggag*attccgttcgagatcctaaaacatctgaaatatta*ggaccgggga*gctgaacaggatcgcaacaca cgtgaagtgttc*ggaccgggga*cctgaccctctaaagccaactaagaggtctttt*ggaccgggga*aa aaatcatacatcaccagatgttgatcttggcgacatttca**ctcgag**cgg. Between segments there is a Gly–Pro–Gly encoding sequence (in italic) predicted to enhance the visability of each possible epitope. A *Bam*HI site and *Xho*I site (in bold) were added to the 5′ and 3′ ends, respectively. And three extra protective nucleotides were then added to both ends. The chimeric gene was synthesized by TaKaRa Biotechnology (Dalian, China).

Expression of synthesized epitopes. The chimeric gene was inserted into the cloning sites (*Bam*HI and *Xho*I) of the expression vector pGEX-6p-1 (Invitrogen). The inserts in three recombinant plasmids were sequenced. The confirmed recombinant plasmid was transformed into *Escherichia coli* strain BL21. And the expressed chimeric peptide with a GST tag was purified by glutathione–Sepharose 4B RediPack Column affinity chromatography according to the manufacturer's instructions (Amersham–Pharmacia Biotech). And the bound fusion protein was eluted with glutathione elution buffer (10 mM reduced glutathione, 50 mM Tris–HCl, pH 8.0) for further analysis.

The six segments and sub-segments derived from S2 and S5 (Table 1) were separately synthesized and cloned into pGEX-6p-1. The recombinant plasmids harboring the individual segment were transformed into *E. coli* BL21. The expressed fusion peptides were used for immune reactivity analysis by ELISA and Western blotting.

Development of monoclonal antibody (McAb). Female, 4- to 6-week-old, BALB/c mice were purchased from Experimental Animal Center of Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. Mice were immunized with 250 μg fusion protein in 0.5 ml emulsion with complete Freund's adjuvant. Two booster injections with the same dose of emulsion each as the first immunization were given at 2-week intervals. One day after the last injection, the spleens were surgically removed from the mice.

Splenocytes were fused with SP2/0 myeloma cells and washed twice with DMEM. Fused cells were then mixed in a 15 ml conical tube and 1 ml of 50% (v/v) PEG was added over 1 min with gentle stirring. The mixture was then centrifuged at 400*g* for 5 min. The fused cell pellet

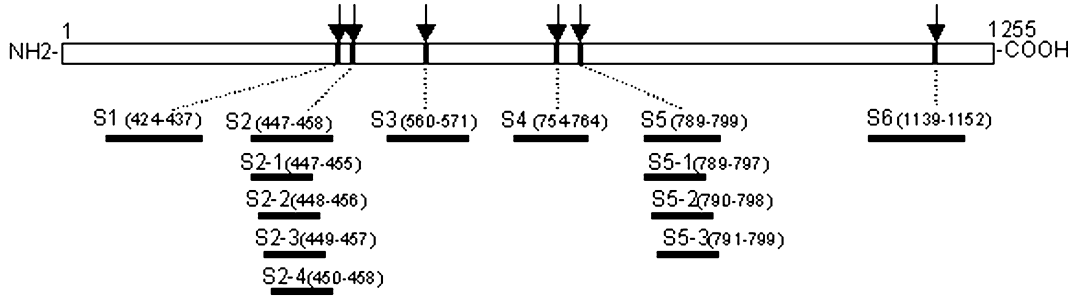

Fig. 1. Schematic diagram of relative location of selected spike protein segments. The box represents the whole length of SARS-CoV spike protein (amino acid residues 1–1255). The arrow refers to the selected peptide. The black bars represent the selected peptide segment and the length and location are indicated. From N to C terminal the selected peptide is named from S1 to S6. Below S2 and S5 there each has a subset bar, which represents a series 9 AA long and 8 AA overlap peptide covering the S2 and S5.

Table 1
Synthesized oligo-nucleotide and deduced peptides

Oligo names	Sequences of synthesized oligoes	AA[a] sequence (location)
S1	5′-*gatcc*aatactaggaacattgatgctacttcaactggtaattataat**taa***c*-3′	NTRNIDATSTGNYN (424–437)
S2	5′-*gatcc*aagcttaggcccttgagagagacatatctaatgtg**taa***c*-3′	KLRPFERDISNV (447–458)
S2-1	5′-*gatcc*aagcttaggcccttgagagagacata**taa***c*-3′	KLRPFERDI (447–455)
S2-2	5′-*gatcc*cttaggcccttgagagagacatatct**taa***c*-3′	LRPFERDIS (448–456)
S2-3	5′-*gatcc*aggcccttgagagagacatatctaat**taa***c*-3′	RPFERDISN (449–457)
S2-4	5′-*gatcc*ccctttgagagagacatatctaatgtg**taa***c*-3′	PFERDISNV (450–458)
S3	5′-*gatcc*gattccgttcgagatcctaaaacatctgaaatatta**taa***c*-3′	DSVRDPKTSEIL (560–571)
S4	5′-*gatcc*gctgaacaggatcgcaacacacgtgaagtgttc**taa***c*-3′	AEQDRNTREVF (754–764)
S5	5′-*gatcc*cctgaccctctaaagccaactaagaggtcttttt**taa***c*-3′	PDPLKPTKRSF (789–799)
S5-1	5′-*gatcc*cctgaccctctaaagccaactaagagg**taa***c*-3′	PDPLKPTKR (789–797)
S5-2	5′-*gatcc*gaccctctaaagccaactaagaggtct**taa***c*-3′	DPLKPTKRS (790–798)
S5-3	5′-*gatcc*cctctaaagccaactaagaggtcttttt**taa***c*-3′	PLKPTKRSF (791–799)
S6	5′-*gatcc*aaaaatcatacatcaccagatgttgatcttggcgacatttca**taa***c*-3′	KNHTSPDVDLGDIS (1139–1152)

Note. The sequences illustrated are all sense strands. At 5′ and 3′ terminal of each sense strand there is a sequence of gatcc and c (in italics), respectively. And at 3′ and 5′ terminal of antisense strand (sequence not shown) there is a sequence of g and gagct, respectively. When the sense and antisense oligo-nucleotides annealed they would form a cohesive *Bam*HI site at 5′ terminus and a cohesive *Xho*I site at 3′ terminus. At the end of peptide encoding gene there is an additional taa sequence to form a stop codon (in bold).

[a] Stands for amino acid.

was re-suspended in DMEM supplemented with 15% FCS, HAT medium, and hybridoma clones were screened by ELISA for McAbs that bound fused protein but not GST protein. Selected clones were subcloned by limiting dilution. Final hybridoma clones were isotyped using an isotyping kit from Roche Diagnostics. Ascites fluids were produced in pristine-primed BALB/c mice. Hybridoma cell lines were grown in RPMI 1640 medium with 10% heat-inactivated FCS. Enzyme-linked immunosorbent assay (ELISA) and Western blot assays were used to measure the activity and specificity of the antibodies.

Enzyme-linked immunosorbent assay. Ninety-six-cell microtiter plates were coated with purified fusion protein or bacterial sonicates in 0.1 M carbonate buffer (pH 9.6) of 10 μg/ml at 4 °C overnight and blocked with 5% skimmed milk for 3 h. After blocking the plates were washed three times with PBST (PBS with 0.1% Tween 20). In binding assay, the plates were incubated with the supernatant of cultured cell or diluted ascites fluids at 37 °C for 1 h followed by washing three times with PBST. Bound antibodies were determined with horseradish peroxidase (HRP)-coupled goat anti-mouse IgG (sigma) and color was subsequently developed with *O*-phenylenediamine dihydrochloride (sigma) and hydrogen peroxide. The reaction was stopped with 2 M H_2SO_4 and the absorbance was measured at 490 nm by microplate autoreader (Bio-Rad).

Western blotting. Cell lyses or proteins were mixed with an equal volume of sample loading buffer (50 mM Tris–HCl, pH 6.8, 100 mM DTT, 2% SDS, 0.1% bromophenol blue, and 10% glycerol), separated by SDS–polyacrylamide gel electrophoresis (PAGE) with 12% polyacrylamide gels. For immunoblotting, proteins were transferred from SDS–polyacrylamide gel to nitrocellulose membrane. Non-specific antibody-binding sites were blocked with 5% skimmed milk in PBS overnight at 4 °C. The membranes were incubated with primary antibody at 37 °C for 1 h. After incubation with primary antibody the membrane was washed three times with PBST (10 min each time). The blot was then probed with a 1:5000 dilution of secondary antibody for 1 h at 37 °C. The secondary antibody is HRP-conjugated goat anti-mouse immunoglobulin G (IgG, sigma) or HRP-conjugated goat anti-human IgG (Beijing Zhongshang Biotechnology) or alkaline phosphatase (AP) conjugated rabbit anti-chicken IgG (Sigma). Then the membrane was washed three times with PBST and developed with the HRP developer solution or AP developer solution.

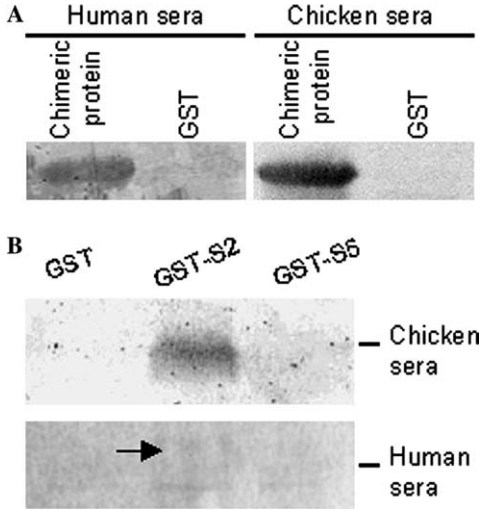

Fig. 2. Western blotting analysis of multi-epitope fusion peptide and epitope S2 and S5. GST fused multi-epitope chimeric protein strongly reactive with convalescent and immunized chicken sera (A); epitope S2 reactive with immunized chicken sera but weakly reactive with convalescent sera (as arrow refer) (B).

Results

The multi-epitope peptide is reactive to sera of SARS patients and immunized chickens

The expressed multi-epitope peptide was strongly reactive to sera from a SARS convalescent patient of

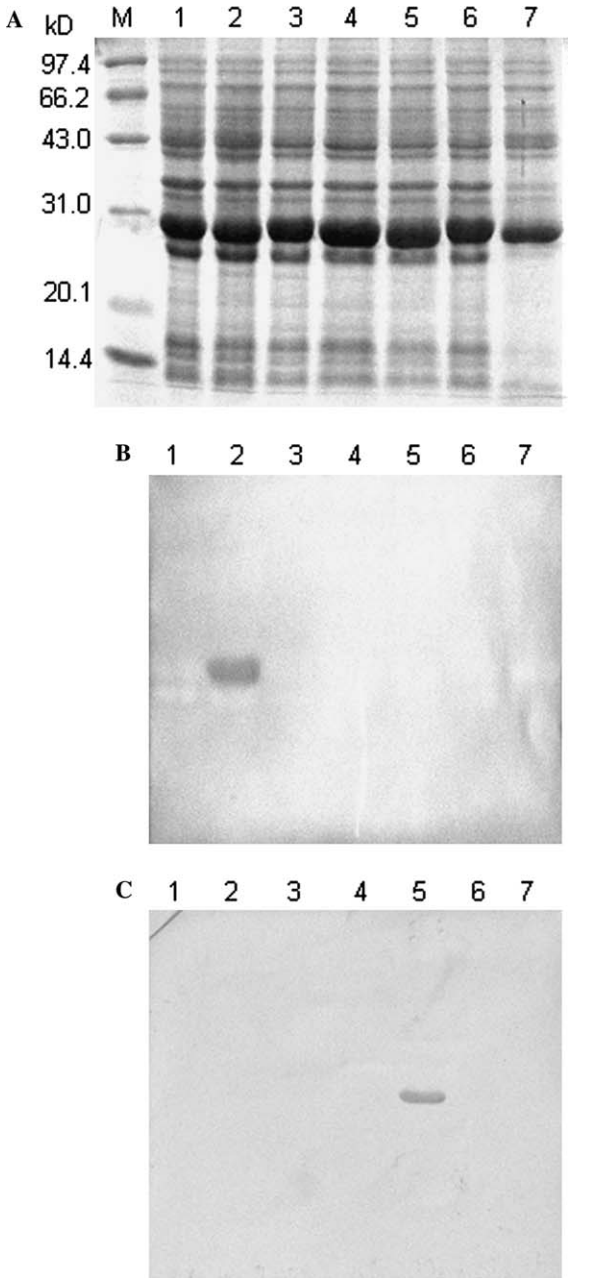

Fig. 3. Western blotting analysis of recombinant fusion peptides with monoclonal antibodies. *E. coli* with plasmids individually expressing S1 (lane 1), S2 (lane 2), S3 (lane 3), S4 (lane 4), S5 (lane 5), S6 (lane 6), and GST (lane 7) were sonicated and the total cell lysates were analyzed by SDS–PAGE (A). After transfer of the gel to nitrocellulose membranes, Western blotting analysis was performed with monoclonal antibodies D3D1 (B) and D3C5 (C), respectively. S2 and S5 were recognized by monoclonal antibodies D3D1 (B) and D3C5 (C), respectively.

Guangdong province and chickens immunized with inactivated vaccine made of SARS-CoV Strain BJ01 in Western blotting (Fig. 2A). Dr. Ming Liao and Dr. Guihong Zhang (Southern China Agricultural University) donated the SARS convalescent sera. And the SARS group of Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences provided immunized chicken sera. The reactivity of six individual fused peptides was also tested by Western blotting with chicken sera and convalescent sera. The result demonstrated that only S2 is reactive, and the signal of S2 to convalescent sera is weak (Fig. 2B).

Two epitopes identified by McAbs

A number of McAbs against the multi-epitope peptide were developed and only two McAbs showed immune reactivity to individually expressed peptides. Epitope S2 (KLRPFERDISNV), locating at 447–458 AA of spike protein, was specifically recognized by McAb D3D1 in Western blotting (Fig. 3B) and ELISA (Fig. 4A), and epitope S5 (PDPLKPTKRSF), locating at 789–799 AA of spike protein, was specifically recognized by McAb D3C5 in Western blotting (Fig. 3C) and ELISA (Fig. 4C).

Minimizing of epitopes recognized by McAbs

In order to further minimize the epitopes (S2 and S5) recognized by McAbs D3D1 and D3C5, four sub-segments derived from S2 and three sub-segments derived from S5 (see Table 1) were synthesized and expressed in a fusion form with GST, respectively. Each sub-segment is 27 nucleotides in length, which encodes 9 amino acids. In ELISA, GST-S2-1 showed strong reaction with McAb D3D1 as GST-S2 did, but GST-S2-2 and GST-S2-3 only showed weak reaction with D3D1, GST-S2-4 completely lost reactivity to the McAb (Fig. 4B). The results demonstrated that S2-1 with 9 amino acids: KLRPFERDI (at 447–455 AA of spike protein) is the core sequence of the epitope S2. During determination the reactivity between the three shortened S5 sub-segments and McAb D3C5 by ELISA, only GST-S5-1 reacted with D3C5 as strong as GST-S5, GST-S5-2 reacted weakly, and GST-S5-3 lost reactivity. These

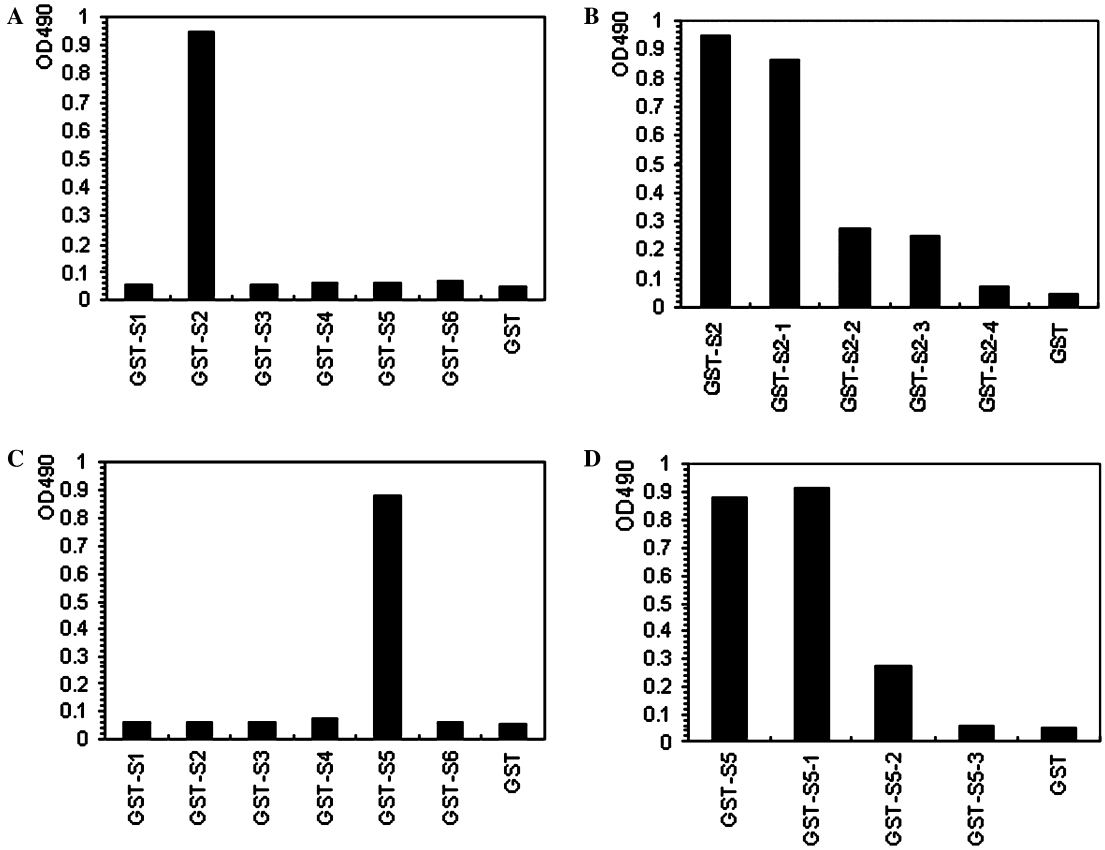

Fig. 4. Detection of recombinant fusion peptides with McAbs by ELISA. Ninety-six-cell microtiter plates were coated with bacterial sonicates containing GST or GST-fused epitopes. After blocking with skimmed milk, monoclonal antibodies D3D1 (A,B) and D3C5 (C,D) were added, and followed by adding secondary antibody HRP-coupled goat anti-mouse IgG. GST and GST-S1 to GST-S6 were detected with McAb D3D1 (A) and D3C5 (C), respectively. GST-S2 and its derivatives were detected with McAb D3D1 (B), and GST-S5 and its derivatives were detected with McAb D3C5 (D).

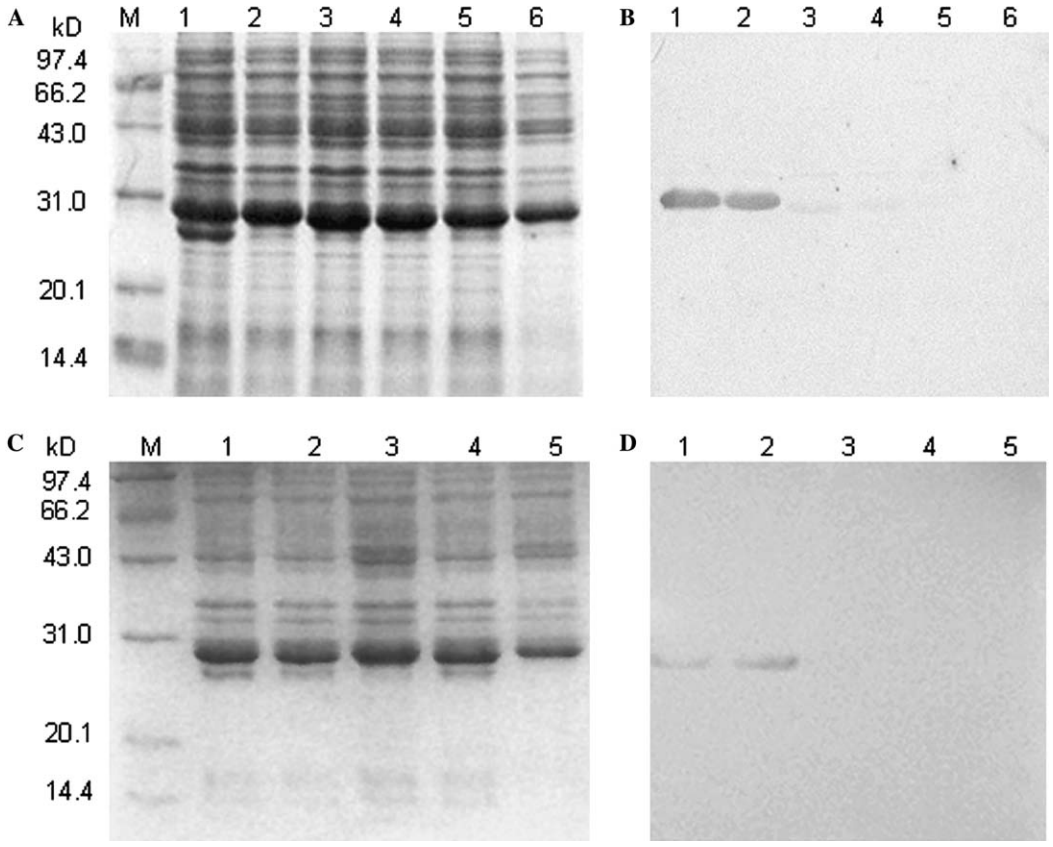

Fig. 5. Detection of S2 or S5 and their derivatives with McAbs by Western blotting. (A) SDS–PAGE analysis of S2 (lane 1) and S2-1 (lane 2), S2-2 (lane 3), S2-3 (lane 4), S2-4 (lane 5), and GST (lane 6). (B) Western blotting was performed on nitrocellulose membrane being transferred from SDS–PAGE gel (A) with McAb D3D1. The number of lane is corresponding to the left figure (A). (C) SDS–PAGE analysis of S5 (lane 1) and S5-1 (lane 2), S5-2 (lane 3), S5-3 (lane 4), and GST (lane 5). (D) Western blotting was performed on nitrocellulose membrane being transferred from SDS–PAGE gel (C) with McAb D3C5. The number of lane is corresponding to the left figure (C). M stands for protein marker.

results clearly demonstrated that S5-1 with 9 amino acids, PDPLKPTKR, located at 789–797 AA of spike protein of SARS-CoV is the core sequence of the epitope S5.

The results from Western blotting analysis were coincidental to that of ELISA (Fig. 5), indicating that the two epitopes recognized by McAbs were all linear epitopes.

Discussion

SARS-CoV is a newly appeared coronavirus. Though tens of the viruses have been isolated and genomically sequenced, little is known about the virus, such as the nature host of the virus, the mechanism of pathogenesis, and the structure and function of proteins encoded by the viral genome. Based on information of other coronavirus, the spike protein plays an important role in cell tropism, specific binding with receptor, cell membrane fusion, and inducing neutralization antibodies [9]. Epitopes are the important antigenic elements of virus structural proteins, which are functional in inducing antibody production and cell-mediated immunity against viruses. Therefore, epitopes are the basis of developing epitope-based vaccines and diagnostic.

In this study, with a panel of McAbs, two epitopes S2 and S5 of SARS-CoV were identified; they located at 447–458 and 789–799 AA on spike protein, respectively. Epitope is the defined region of an antigen reactive with the corresponding antibody. There are linear and conformational epitopes, linear epitopes are short stretches of the primary structure of the protein and consist of 6–9 or more continuous amino acid residues. Conformational epitopes consist of several amino acid residues that are discrete in the primary sequence but assemble to form an antigenic determinant on the tertiary structure of the native protein. In this study, McAbs D3C5 and D3D1 effectively detected S5 and S2, respectively, in denatured gels (SDS–PAGE). The results indicated that the epitopes S2 and S5 recognized by D3C5 and D3D1 are linear epitopes. Further we examined the reactivity of two McAbs to the 9 AA length subsets of S5 and S2, the precise locations of two epitopes were confirmed.

In Western blotting analysis, epitope S2 was reactive with immunized chicken sera. This indicated that epitope S2 is a major epitope in chicken sera. In this report epitope S2 is weakly reactive with convalescent sera. Whether epitope S2 is a major epitope in human needs further investigation. We did not test the reactivity of epitope S2 to more convalescent sera.

By bio-informatics analysis, the amino acids from 1 to 769 formed the spherical head of the spike protein. And the rest of the segment from 770 to 1225 formed the stem portion and trans-membrane structure of spike protein. So the epitope S5 located in stem portion and epitope S2 located in spherical head of the spike protein. Recently, Li et al. [14] have identified that angiotensin-converting enzyme 2 (ACE2) is a functional receptor for the SARS coronavirus. Following this report, Xiao et al. [15] cloned, expressed, and characterized the full-length and various soluble fragments of the S glycoprotein of SARS-CoV (Tor2 isolate). They found that fragments containing the N-terminal amino acid residues 17–537 and 272–537 but not 17–276 bound specifically to Vero E6 cells and purified soluble receptor ACE2. And they confirmed that the receptor-binding domain is located between amino acid residues 303 and 537. Further more, Wong et al. [16] recently reported that a 193-amino-acid fragment of the S protein (residues 318–510) bound ACE2 more efficiently than did the full S1 domain (residues 12–672). And the residue 454 (aspartic acid) is unmutationable in the receptor binding reactivity. The epitope S2 (447–458) we identified here locates spang in receptor binding domain. So our results could provide useful information for further analyzing the function of spike protein and prophylaxis of SARS.

Acknowledgments

This work was partially supported by a Heilongjiang postdoctoral grant. We greatly appreciate all SARS group colleagues of Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences for providing inactived SARS-CoV sonicates antigen and chicken sera. We thank Dr. Ming Liao and Dr. Guihong Zhang of Southern China Agricultural University for providing SARS convalescent sera.

References

[1] J.S. Peiris, S.T. Lai, L.L. Poon, Y. Guan, Y.L. Yam, W. Lim, J. Nicholls, W.K. Yee, W.W. Yan, M.T. Cheung, V.C. Cheng, K.H. Chan, D.N. Tsang, R.W. Yuang, T.K. Ng, K.Y. Yuen, SARS study group, coronavirus as a possible cause of severe acute respiratory syndrome, Lancet 361 (2003) 1319–1325.

[2] C. Drosten, S. Gunther, W. Preiser, S. van der Werf, H.R. Brodt, S. Becker, H. Rabenau, M. Panning, L. Kolesnikova, R.A. Fouchier, A. Berger, A.M. Burguiere, J. Cinatl, M. Eickmann, N. Escriou, K. Grywan, S. Kramme, J.C. Manuguerra, S. Muller, V. Rickerts, M. Sturmer, S. Vieth, H.D. Klenk, A.D. Osterhaus, H. Schmits, H.W. Doerr, Identification of a novel coronavirus in patients with severe acute respiratory syndrome, N. Engl. J. Med. 348 (2003) 1967–1976.

[3] T.G. Ksiazek, D. Erdman, C.S. Goldsmith, S.R. Zaki, T. Peret, S. Emery, S. Tong, C. Urbani, J.A. Comer, W. Lim, P.E. Rollin, S.F. Dowell, A.E. Ling, C.D. Humphrey, W.J. Shieh, J. Guarner, C.D. Paddock, P. Rota, B. Fields, J. DeRisi, J.Y. Yang, N. Cox, J.M. Hughes, J.W. LeDuc, W.J. Bellini, L.J. Anderson, SARS working group, a novel coronavirus associated with severe acute respiratory syndrome, N. Engl. J. Med. 348 (2003) 1953–1966.

[4] E.D. Qin, Q.Y. Zhu, M. Yu, B.C. Fan, G.H. Chang, B.Y. Si, B.A. Yang, W.M. Peng, T. Jang, B.H. Liu, Y.Q. Deng, H. Liu, Y. Zhang, C.E. Wang, Y.Q. Li, Y.H. Gan, X.Y. Li, F.H. Lu, G. Tan, W.C. Cao, R.F. Yang, J. Wang, W. Li, Q.F. Wu, Y. Li, Z.Y. Xu, W. Lin, W.J. Chen, H.T. Chen, L.J. Cong, F. Chen, Y.J. Deng, L. Fang, Y.Q. Huang, X.G. Huang, Y.J. Han, J.F. Hu, W. Hu, J. Ji, Y.Z. Jiao, M. Lei, C.F. Li, G.Q. Li, S.T. Li, S.L. Li, H.H. Li, W.J. Li, D.W. Li, C.J. Li, J. Liu, Y. Liu, H. Lu, Q.H. Qi, H.O. Qin, J.P. Shi, Y.Q. Sun, L. Tang, W. Tong, Z.Z. Tong, X.J. Tian, J. Wang, Z.Q. Wang, J.J. Wang, Y. Xi, J. Xu, L.H. Yang, C. Ye, J. Ye, B. Zhang, X.Q. Zhao, F. Zhang, J.G. Zhang, Z.J. Zhang, H.Q. Zhang, Y.L. Zhang, B. Liu, C.Q. Zeng, S.G. Li, W. Gane Ka-Shu, X.H. Tan, S.Q. Liu, W. Dong, J. Wang, J. Yu, H.M. Yang, A complete sequence and comparative analysis of a SARS-associated virus (Isolate BJ01), Chin. Sci. Bull. 48 (2003) 941–948.

[5] P.A. Rota, M.S. Oberste, S.S. Monroe, W.A. Nix, R. Campagnoli, J.P. Icenogle, S. Penaranda, B. Bankamp, K. Maher, M.H. Chen, S.X. Tong, A. Tamin, L. Lowe, M. Frace, J.L. DeRisi, Q. Chen, D. Wang, D.D. Erdman, T.C.T. Peret, C. Burns, T.G. Ksiazek, P.E. Rollin, A. Sanchez, S. Liffick, B. Holloway, J. Limor, K. McCaustland, M. OlsenRassmussen, R. Fouchier, S. Gunther, A.D.M.E. Osterhaus, C. Drosten, M.A. Pallansch, L.J. Anderson, W.J. Bellini, Characterization of a novel coronavirus associated with severe acute respiratory syndrome, Science 300 (2003) 1394–1399.

[6] M.A. Marra, S.J. Jones, C.R. Astell, R.A. Holt, A. Brooks-Wilson, Y.S. Butterfield, J. Khattra, J.K. Asano, S.A. Barber, S.Y. Chan, A. Cloutier, S.M. Coughlin, D. Freeman, N. Girn, O.L. Griffith, S.R. Leach, M. Mayo, H. McDonald, S.B. Montgomery, P.K. Pandoh, A.S. Petrescu, A.G. Robertson, J.E. Schein, A. Siddiqui, D.E. Smailus, J.M. Stott, G.S. Yang, F. Plummer, A. Andonov, H. Artsob, N. Bastien, K. Bernard, T.F. Booth, D. Bowness, M. Czub, M. Drebot, L. Fernando, R. Flick, M. Garbutt, M. Gray, A. Grolla, S. Jones, H. Feldmann, A. Meyers, A. Kabani, Y. Li, S. Normand, U. Stroher, G.A. Tipples, S. Tyler, R. Vogrig, D. Ward, B. Watson, R.C. Brunham, M. Krajden, M. Petric, D.M. Skowronski, C. Upton, R.L. Roper, The Genome sequence of the SARS-associated coronavirus, Science 300 (2003) 1399–1404.

[7] Y. Guan, B.J. Zheng, Y.Q. He, X.L. Liu, Z.X. Zhuang, C.L. Cheung, S.W. Luo, P.H. Li, L.J. Zhang, Y.J. Guan, K.M. Butt, K.L. Wong, K.W. Chan, W. Lim, K.F. Shortridge, K.Y. Yuen, J.S. Peiris, L.L. Poon, Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China, Science 302 (2003) 276–278.

[8] M. Eickmann, S. Becker, H.D. Klenk, H.W. Doerr, K. Stadler, S. Censini, S. Guidotti, V. Masignani, M. Scarselli, M. Mora, C. Donati, J.H. Han, H.C. Song, S. Abrignani, A. Covacci, R. Rappuoli, Phylogeny of the SARS coronavirus, Science 302 (2003) 1504–1505.

[9] T.M. Gallagher, M.J. Buchmeier, Coronavirus spike proteins in viral entry and pathogenesis, Virology 279 (2001) 371–374.

[10] J. Kyte, R.F. Doolittle, A simple method for displaying the hydropathic character of a protein, J. Mol. Biol. 157 (1982) 105–132.

[11] E.A. Emini, J.V. Hughes, D.S. Perlow, J. Boger, Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide, J. Virol. 55 (1985) 836–839.

[12] B.A. Jameson, H. Wolf, The antigenic index: a novel algorithm for predicting antigenic determinants, Comput. Appl. Biosci. 4 (1988) 181–186.
[13] P.Y. Chou, Prediction of protein structural classes from amino acid composition, in: Prediction of Protein Structure and the Principles of Protein Conformation, Plenum Press, New York, 1990, pp. 549–586.
[14] W. Li, M.J. Moore, N. Vasilieva, J. Sui, S.K. Wong, M.A. Berne, M. Somasundaran, J.L. Sullivan, K. Luzuriaga, T.C. Greenough, H. Choe, M. Farzan, Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus, Nature 426 (2003) 450–454.
[15] X. Xiao, S. Chakraborti, A.S. Dimitrov, K. Gramatikoff, D.S. Dimitrov, The SARS-CoV S glycoprotein: expression and functional characterization, Biochem. Biophys. Res. Commun. 312 (2003) 1159–1164.
[16] S.K. Wong, W. Li, M.J. Moore, H. Choe, M. Farzan, A 193-amino-acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2, J. Biol. Chem. 279 (2004) 3197–3201.