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SARS Patients-derived Human Recombinant Antibodies to S and M Proteins Efficiently Neutralize SARS-Coronavirus Infectivity[1]

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Objective To develop a specific SARS virus-targeted antibody preparation for emergent prophylaxis and treatment of SARS virus infection. **Methods** By using phage display technology, we constructed a naive antibody library from convalescent SARS patient lymphocytes. To obtain the neutralizing antibody to SARS virus surface proteins, the library panning procedure was performed on purified SARS virions and the specific Fab antibody clones were enriched by four rounds of repeated panning procedure and screened by highthroughput selection. The selected Fab antibodies expressed in the periplasma of *E. coli* were soluble and further purified and tested for their binding properties and antiviral function to SARS virus. The functional Fab antibodies were converted to full human IgG antibodies with recombinant baculovirus/insect cell systems and their neutralizing activities were further determined. **Results** After four rounds of the panning, a number of SARS-CoV virus-targeted human recombinant Fab antibodies were isolated from the SARS patient antibody library. Most of these were identified to recognize both natural and recombinant SARS spike (S) proteins, two Fab antibodies were specific for the virus membrane (M) protein, only one bound to SARS-CoV nucleocapsid protein. The SARS-CoV S and M protein-targeted Fab or IgG antibodies showed significant neutralizing activities in cytopathic effect (CPE) inhibition neutralization test, these antibodies were able to completely neutralize the SARS virus and protect the Vero cells from CPE after virus infection. However, the N protein-targeted Fab or IgG antibodies failed to neutralize the virus. In addition, the SARS N protein-targeted human Fab antibody reacted with the denatured N proteins, whereas none of the S and M protein specific neutralizing antibodies did. These results suggested that the S and M protein-specific neutralizing antibodies could recognize conformational epitopes which might be involved in the binding of virions to cellular receptors and the fusion activity of the virus. **Conclusion** The SARS-CoV spike protein and membrane proteins are able to elicite efficient neutralizing antibodies in SARS patients. The neutralizing antibodies we generated in this study may be more promising candidates for prophylaxis and treatment of SARS infection.

Key words: SARS-CoV; Phage display; Human antibody

INTRODUCTION

The outbreak of severe acute respiratory syndrome (SARS) has deadly threatened public heath in the world, especially in China. A novel coronavirus (SARS-CoV) is etiologically linked to the outbreak of SARS after a line of SARS-CoV evidence was found in SARS patients throughout the world[1-2]. SARS-CoV virus is a novel RNA virus member in the family of Nidovirale, with the viral genome of 29 727 nucleotides in length and 11 open reading frames[3]. As a typical coronavirus, SARS-CoV has

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the gene order of 5′-replicase (rep), spike (S), envelope (E), membrane (M) and nucleocapsid (N) as well as short untranslated region. The S, E, M, and N open reading frames located down stream of rep gene are predicted to encode the viral structural proteins. The S and M proteins of coronavirus are assembled on the surface of the virion for binding to receptors on host cells and the membrane fusion, thus also containing important virus-neutralizing epitopes and eliciting neutralizing antibody responses.

An important factor in the valuation of virus vaccine candidate is the ability to elicit neutralizing antibodies. Neutralizing antibodies have been shown to play a major role in resistance to disease in humans as well as in protection experimental animals against infection[4-5]. Also SARS patients remain viremic during acute phase of the disease as evidenced by the virus genes from SARS patient blood by RT_PCR analysis and isolation of the virus from blood in multiple cell lines[1,6-7]. The reports promoted an idea to develop specific SARS virus-targeted human neutralizing antibodies for emergent prophylaxis and treatment of SARS virus-infection. Antibody engineering technologies have been greatly improved for the fast development and large production of human antiviral neutralizing antibodies.

We have approached the issue of fast development of neutralizing antibodies against SARS-CoV by using phage display and antibody engineering platform technology. Twenty-five human monoclonal antibodies have been isolated by screening SARS patients-derived antibody library on purified SARS-CoV virions. Some of them have neutralizing activities to SARS-CoV infection in Vero-cells. The results provide a new prospect for the development of therapeutic antibodies to prevent SARS outbreak.

MATERIALS AND METHODS

Viruses, Cells and Lymphocytes Isopropiolactone in SARS Patients

The SARS virus strain (SARS-CoV CV8) was isolated from the blood of 6 SARS patients and propagated on Vero cells (ATCC CRL 1586)[8]. Vero cells were infected with1000 TCID50 SARS-Cov CV8 and cultured for 3-4 days. The infected medium supernatant was harvested and the virus was inactivated by adding 1:4000 β-propiolactone at 4℃ for 2 days, and further clarified by centrifugation at 10 000×g for 30 min at 4℃ and filtered through a 0.22 mm filter. Virus particles were pelleted through a 25% sucrose cushion (in TNE buffer: 10 mmol/L Tris/HCl, pH 7.5, 100 mmol/L NaCl, 1 mmol/L EDTA) in an ultracentrifuge at 72 000xg for 4 h at 4℃ using a SW-41 rotor. The virus pellets were resuspended in 500 μL of TNE buffer per 240 mL of culture supernatant. The identity of the virus was tested by ELISA and SDS-PAGE analysis. The human lymphocytes used for RNA extraction were isolated from 6 SARS convalescence patients in Beijing ranging from 25 to 36 days after onset of SARS.

Construction, Panning and Screening of Human Antibody Library

Six SARS patient blood lymphocytes were purified on a Ficoll gradient. Total cellular RNA was extracted from pelleted lymphocytes using Trizol reagent (GIBCO BRL, Gaithersburg, MD) and cDNA was synthesized by oligo (dT) primers and reverse transcriptase. The heavy chain Fd and light chain genes were amplified from the cDNA by polymerase chain reaction (PCR) and sequentially cloned into the phagemid vector pComb3 as described elsewhere[9]. The human antibody phage library was constructed after transformation of *E. coli* followed by the rescue of helper phage, and selected by panning on purified SARS-CoV virions as previously described[8]. After three or four rounds of panning, the human Fab antibody genes were expressed in *E. coli* and Fabs were screened by ELISA and immune fluorescence assay (IFA) as described below.

Sequence Analysis of Variable Regions of Fabs

Nucleic acid sequencing was performed with the ABI PRISM dye terminator cycle sequencing ready reaction kit and the primers: 5′CCGCGGTGGCGGCCGCAA3′ for the VH gene of heavy chains and the primer 5′AAACTA GCTAGTCGCCAAGGA3′ for the VL of the light chains. Sequences were analyzed by aligning to human immunoglobulin sequences present in Genebank and the CDR regions were determined with the V-Base program (http://www.mrc-cpe. cam. ac. uk/vbase)

Conversion of Fab Antibody to IgG Antibody

The VH and VL or Fab genes of 5 human Fabs selected from the phage library were cloned into IgG expression cassette vectors pAc-L-Fc, as described previously, such that in-frame ligations to a human Fc region were obtained[10]. The resulting expression vector DNAs containing heavy and light chain genes were transfected into SF9 insect cells, and recombinant baculoviruses were prepared by homologous recombination using the Baculo-Gold transfection kit (Pharmingen, USA) according to

the manufacturer's instructions. The expression and purification of IgG antibodies were verified by IFA, ELISA and SDS-PAGE as previously described[10].

ELISA Analysis of Antibody Binding

ELISA was performed for screening the crude Fab or IgG antibody preparations, determining the binding avidities of Fab or IgG antibodies to hantaviruses. Direct ELISA was performed by coating each well of a 96 well plate with approximately 0.5-1 µg of sucrose gradient- purified SARS-CoV diluted in 0.01 mol/L sodium carbonate buffer, pH 8.6 or purified recombinant SARS-CoV N protein (unpublished data). The plates were blocked with 4% skim milk in PBS, then the crude or purified Fab or IgG antibody preparations were added. Wells were then washed and incubated with HRP-conjugated anti-human Fab or anti-human Fc (Sigma) followed by TMB substrate for color development.

Confocal Immunofluorescence Assay (IFA)

SARS-CoV antigen slides were prepared in our laboratory using standard protocol. Briefly, Vero cells were infected with SARS-CoV for 3 days. The infected cells were digested with trypsin-EDTA (GIBCO-BRL) and washed with PBS once, then resuspended in PBS at the concentration around 1×10^6 cells/mL. The cells were fixed on 10- or 12-well slides with methanol at room temperature for 0.5 h, dried and kept at –70℃ for next steps. In addition to these slides prepared with authentic SARS-CoV antigens, we prepared IFA slides from recombinant baculovirus-infected SF9 cells for SARS-CoV M protein screening. The crude *E.coli*-expressed Fab antibodies and SF9 cells-expressed IgG antibodies or purified-IgG antibodies were incubated with the slides described above at 37℃ for 30 min. Bound antibodies were detected with FITC conjugated anti-human Fab and human Fc antibodies under confocal Immunofluorescence microscope.

Characterization of Viral Protein Binding Specificities of the Antibodies

To characterize the binding properties of SARS-CoV specific human antibodies to viral proteins, Western-blots and immunoprecipitation of SARS-CoV viral proteins were performed according standard operation protocols as described in the text book of molecular cloning. For SDS-PAGE and Western-blot analysis, the purified SARS-CoV virus particle antigen was applied for SDS-PAGE. After electrophoresis, the gel was blotted onto a nitrocellulose membrane using a standard Western-blot protocol. The membranes were stained with SARS-patient sera, normal human sera at 1:50 dilution, or the Fab and IgG antibodies at 1 g/mL. HRP conjugated anti- human IgG antibody was used for the detection.

Immunoprecipitation of SARS-CoV viral proteins was performed with the immunoprecipitation kit (Roche Diagnostics) according to the manufacturer's instruction. Confluent monolayers of Vero cells in T25 flasks were infected with 1000 TCID50 SARS-CoV strain CV8 for 2 days. The cells were lysed on ice with 0.8 mL of cell lysis buffer provided by the manufacturer and centrifuged to remove the nuclei. Viral proteins were labelled by biotin-7-NHS and immuno-precipitated by mixing 100 µL of cell lysate with 100 µL of SARS patient sera, normal human sera or the crude *E. coli*- expressed Fab antibody preparations. After incubation at 4℃ overnight, protein L-sepharose 4 B (Piece, USA) for Fab antibodies was added individually and the mixture was roked at 4℃ for 3 h. The beads with immune-precipitated proteins were washed three times with lysis buffer provided in the kit resuspended in 2X regular SDS-PAGE loading buffer and analyzed by SDS-PAGE as standard protocol at the gel concentrations of 10% (For S protein) and 15% (For M protein). The precipitated bands were tested by Western-blot assay according to the manufacturer's instructions.

Virus Neutralization Assay

The neutralization activity of SARS patients-derived antibodies was measured by cytopathic effect (CPE) inhibition neutralization assay. Briefly, Vero-E6 cells were cultured in 24-well plates till to a monolayer. The purified human recombinant Fab and IgG antibodies were standardized to 100 µg/mL and serially diluted in four-fold increments. One hundred µL of each antibody dilution was incubated with 100 µL of 100 TCID50 SARS-CoV solution at 4℃ overnight. Virus-antibody mixtures were applied to the cell monolayers in a 24-well plate for 4 wells per test sample and incubated at 37℃ for 2 h, then the mixtures were removed and replaced by fresh EMDM (Invitrogen), incubated at 37℃ for about 3-5 days till 100% CPE appeared in virus positive control wells. The neutralizing antibody activity was defined by 50% inhibition of CPE as compared to the control.

RESULTS

Screening of SARS-CoV Specific Antibodies From SARS Patient Library

A combinatorial antibody library was prepared from 6 selected convalescent SARS patients possessing high level of serum IgG antibodies to both purified SARS-CoV virions and recombinant SARS-CoV N protein. The complexity of the library deduced from the colonies of transformation after cloning of heavy chain Fd into light chain library was determined to be 2.2×106. After panning of the library on purified SARS-CoV virions, 192 wells of Fab antibody clones taken from the cultured bacterial medium of 96 wells were finally screened by ELISA for testing their nature and binding properties respectively. After three rounds of panning, 46 human Fab positive clones were selected from 96-well expressions (Fig. 1A), 7 SARS-CoV- targeted specific Fab clones of them were determined by immunoflurescsence assay (IFA) (data not shown). After four rounds of panning, nearly 100% human Fab positive clones were selected from 96-well expressions (Fig. 1B) and 52 SARS-CoV-targeted specific Fab clones were determined (Fig. 2A). However, only 9 Fab clones were detected to react with SARS-CoV recombinant N protein, which was previously expressed in *E. coli* as a His-tag fusion protein (Fig. 2B) (unpublished data). The enrichments of SARS-CoV specific Fab clones were efficiently approached by panning on the native SARS-CoV virus particles .

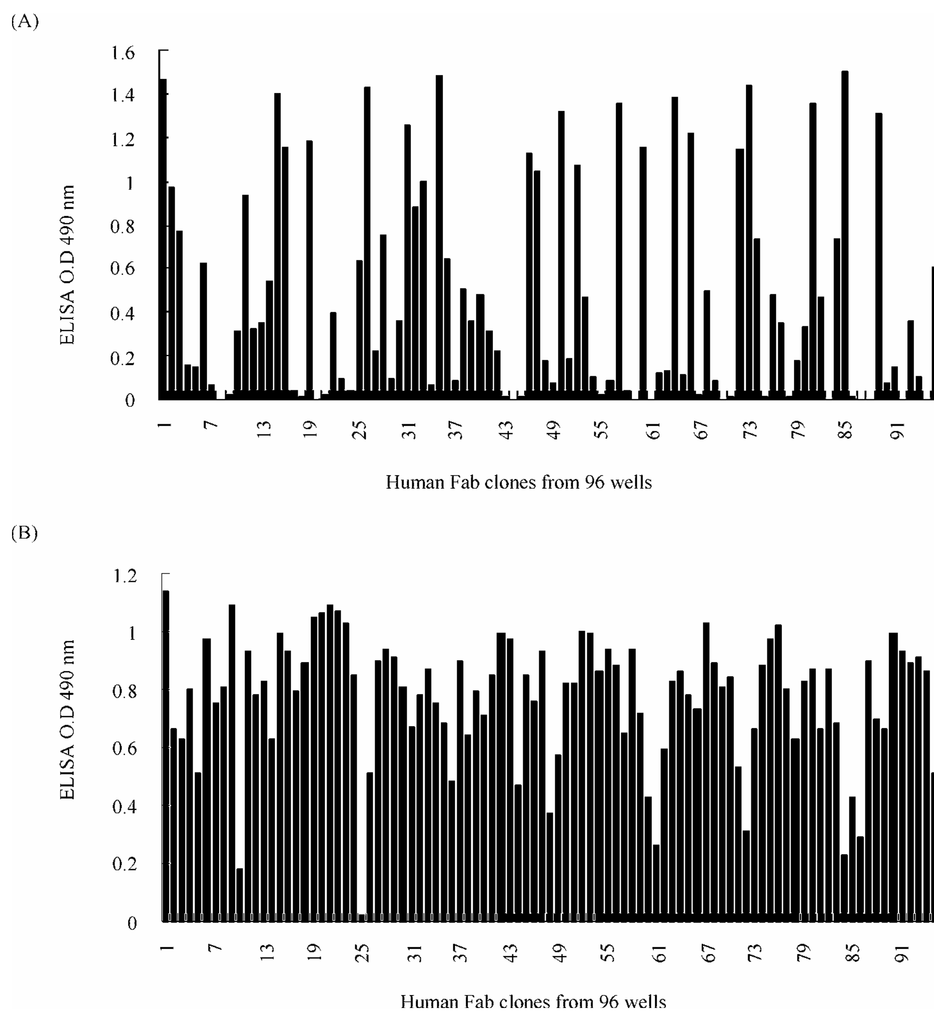

FIG. 1. Enrichment of SARS patients-derived antibody library by panning on SARS-CoV virions. Human Fab antibodies were detected by ELISA after the third round (A) and the fourth round (1B) of panning and screening.

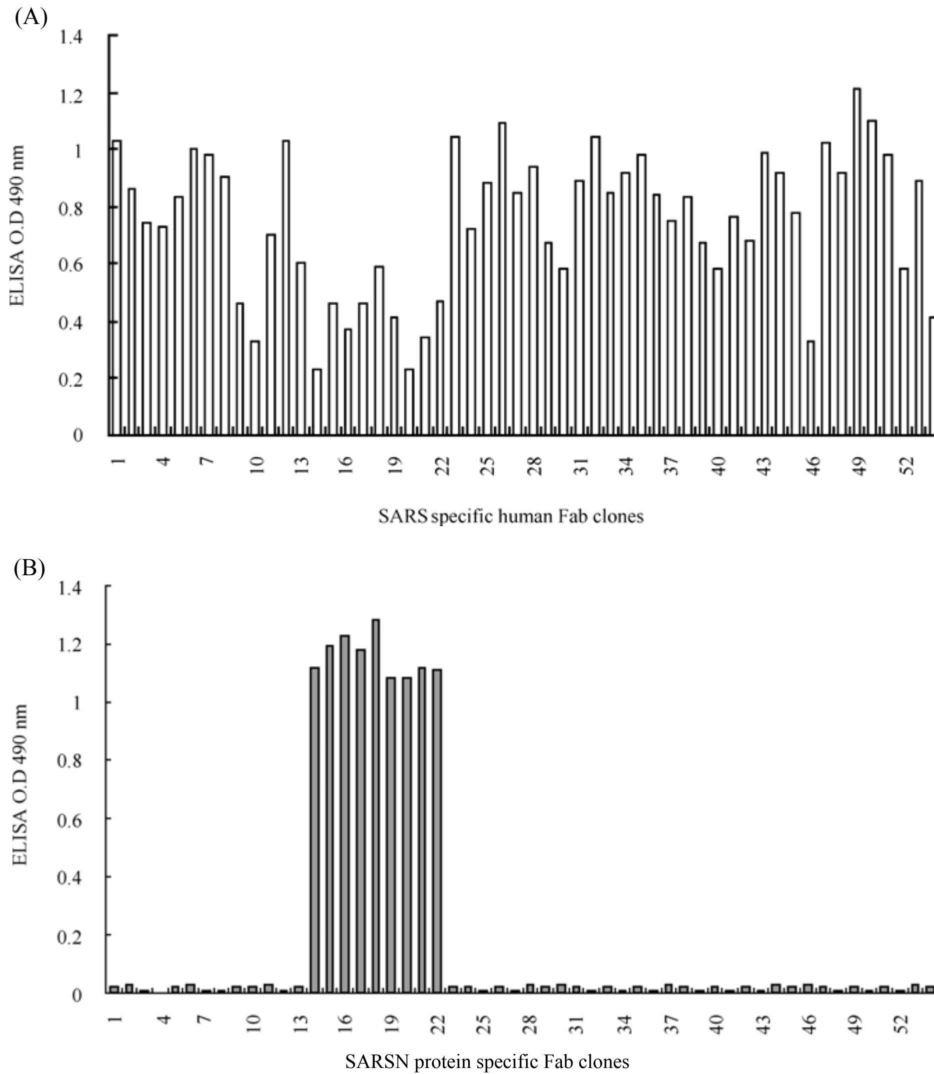

FIG. 2. Specific human Fab antibodies detected by ELISA with coating SARS-CoV virus (A) and recombinant SARS-CoV N protein (B) on 96-well plates.

Binding Specificity of Human-derived Antibodies to SARS-CoV

To confirm the binding specificity of human recombinant antibodies to SARS-CoV virus, the clarified supernatants from the bacterial lysates containing human Fab antibodies were used to further characterize above selected SARS-CoV specific Fab clones IFA and Western blot analysis. IFA was performed by incubating the recombinant Fab antibodies to the IFA slides and stained with IFTC conjugated anti-human Fab antibodies. All the selected human Fab clones were able to bind to SARS-CoV virus. As shown in Fig. 3, two kinds of immunoflurescence reaction pattern were observed under confocal immunoflurescence microscope. The pattern obtained with SARS-CoV N specific human Fab clones represented by clone SARSFab 20 showed a typically cytoplasmic diffuse distribution and the "donut-like" aggregates as illustrated in Figs. 3A and 3B. A number of isolated human Fab clones that were not able to bind to recombinnat SARS-N protein in ELISA showed a different pattern compared with N specific human clones. As shown in Figs. 4C and 4D, the pattern obtained with S and M proteins-reacted clones represented by Fab clone SFab 58 showed a reticular secretary or endoplasma reticular (ER) pathway pattern as illustrated in Figs. 3C and 3D.

Genomic Information of Human Antibodies to SARS-CoV

The genes encoding the variable regions of light (VL) and heavy (VH) chains of 55 selected SARS-CoV specific Fab antibody clones were sequenced

FIG. 3. Confocal immunoflurescent assay of human Fab antibodies to SARS-CoV. The pattern obtained with N specific human Fab clones represented by clone SARSFab 20 shows a typically cytoplasmic diffuse distribution (A, B). The pattern obtained with S and M protein reacted clones represented by Fab clone SFab 58 shows a reticular secretary or endoplasma reticular (ER) pathway pattern (C, D).

and analyzed. The deduced amino acid sequences were analyzed and aligned using DNAsis software. Fig. 4 shows the final comparisons of the deduced amino acid sequences of the CDR3 region of SARS-CoV specific Fab antibody VH and VL genes. Twenty five different VH sequences and 26 different VL sequences were found, and resulted in the 25 different VH/VL combinations of the SARS-CoV-targeted human Fab clones. The antibody H chains were derived from three antibody gene families including VH3, VH6 and VH1 types, and the antibody light chains were major derived from kappa gene families included VKI, VKII and VKIII types according to Kabat sequences and V-Base data. None of light chains of above isolated clones showed lambda (λ) type, 9 of recombinant SARS N protein binding Fab antibodies shared identical light and heavy chains (As shown for clones SFab20) which were classified in independent VKII and VHI families compared with others. The high homology indicated that these clones were derived from a single B-cell clone and might act as one of the SARS patient dominant antibodies to SARS N protein after SARS-CoV infection. For those none SARS N protein binding but SARS virus specific Fab clones as shown in Fig.4, the heavy chain (VH) sequences could be organized into 4 groups including 22 distinct types mainly distributed in VH3 gene groups beginning with SARSFab1. The high diversity was found in CDR3 region between each group. The light

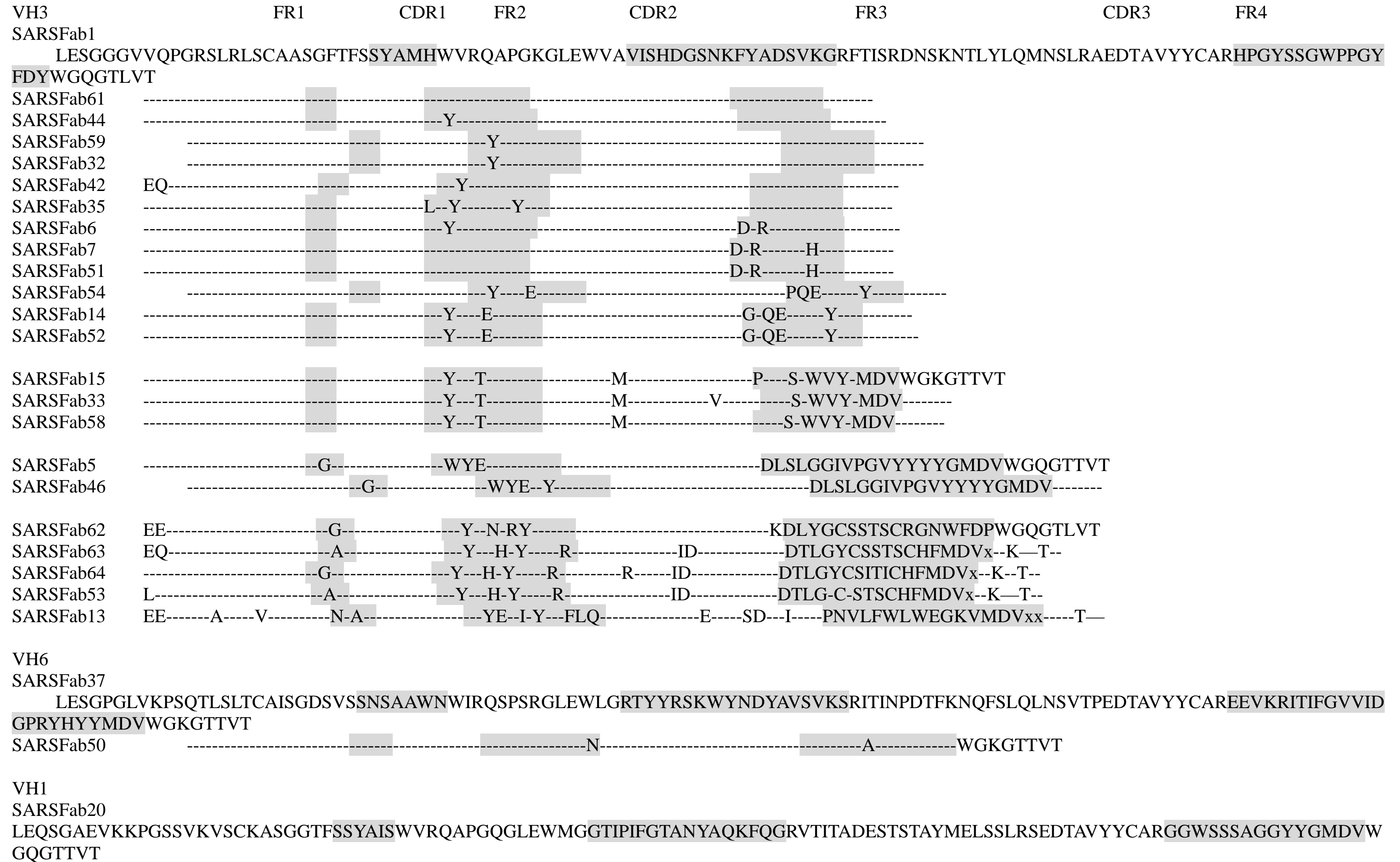

FIG. 4A

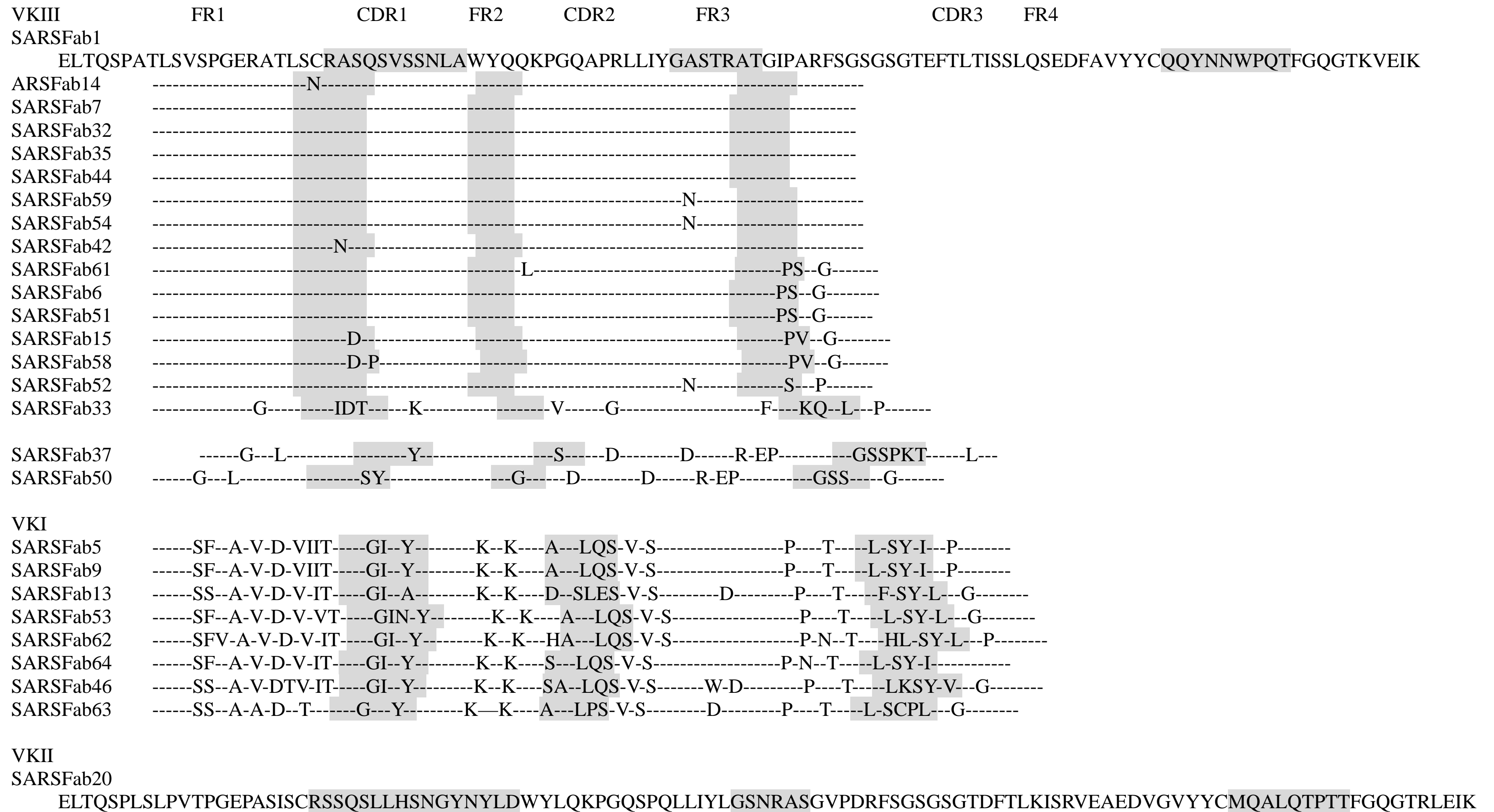

FIG. 4B

FIG. 4. Deduced amino acid sequences of the heavy chain (A) and light chain (B) variable regions of human antibodies to SARS-CoV S (VH3 vs VK3 and VK1), M (VH6 vs VK3) and N (VH1 vs VKII) proteins. The dashed line indicates the same amino acid in comparison with the amino acid sequence of first line.

chain (VK) sequences could be defined into two gene families VKI and VKIII beginning with SFab1 and SFab5, 25 light chain sequences were organized.

Conversion of Human Fab Antibody to Full Length IgG Antibody

Six clones (SFab 59, 58, 15, 63, 50, 20) showing the strongest binding to native SARS-CoV viruse but with different genetic information at their CDRs of the VH and VL genes, were further converted into intact human IgG1 antibodies in the baculovirus expression cassette vector system as previously described[10]. The recombinant human IgG molecules were purified by affinity chromatography and the typical tetramer structure of complete IgG was determined by SDS-PAGE assay under reducing and non-reducing conditions as compared with commercially purchased human reference IgG preparations (Sigma, USA) (data not shown).

Binding of Human Recombinant Antibodies to SARS-CoV Viral Proteins

Further investigation of the viral protein binding properties of the antibodies was first carried out by Western blot analysis. As shown in Fig. 5 A, only SARS N protein specific Fab clones or IgG antibody represented by SFab 20 and SARS patient convalescent sera recognized the N proteins, indicating that the N targeted human monoclonal antibody could recognize a liner epitope on the N protein, the rest of human Fab clones or IgG antibodies, were not able to recognize the N protein after SDS-PAGE denaturation (data not shown). SARS patient sera were also not able to bind to other SARS viral proteins except for the N protein. Therefore immunoprecipitation test was further performed. SARS viral proteins from SARS virus-infected Vero cell lysates were incubated with human Fab or IgG antibodies or SARS patients sera, specific viral proteins could be precipitated. As shown in Fig. 5B, SFab 15, 58, 59, 63-IgG were able to precipitate a band at mobility about 180Kd in 10% SDS-PAGE gel, in comparison with SARS patient sera (Fig. 5B, line HP) which also precipitated the same mobility band. It is more likely as SARS spike protein according to the deduced size from the virus genomic information[3]. However, the SFab50-IgG antibody reacted with a band at mobility about 25Kd in 15% SDS-PAGE gel (Fig. 5C). The antibody could target SARS-CoV M protein by strongly reacting with recombinant baculovirus- expressed M protein in IFA (data not shown).

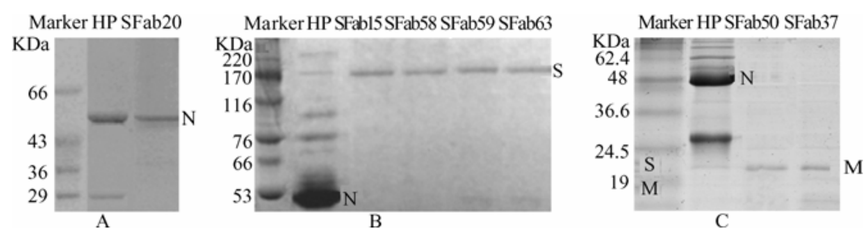

FIG. 5. Western-blot detection and immunoprecipitation of SARS-CoV viral N(A), spike (B), and M (C) proteins with human Fab antibodies.

Neutralization of SARS-CoV Infection With Purified Human IgG Antibodies

To determine whether SARS patients-derived human monoclonal antibodies could efficiently neutralize the SARS-CoV infectivity *in vitro*, the purified IgG antibodies respectively reacted with SARS-CoV S, M and N proteins were filtered through sterilized filters and further applied to cytopathic effect (CPE) inhibition neutralization tests. As shown in Table 1, 3 S-targeted antibodies SFab15IgG, Fab58IgG, SFab59IgG could completely neutralize 100 TCID 50 SARS-CoV at the concentration of 0.1-0.3 μg and 50% neutralizing at 0.02-0.07 μg. SFab63IgG however failed to neutralize the virus infection. Sfab50IgG also showed a significant neutralizing activity at the concentration of 0.5 μg. The N protein-targeted Fab or IgG antibodies failed to neutralize the SARS-CoV virus. Fig. 6 shows the morphology of Vero cells after infection and the antibody neutralization. SARS-CoV infected Vero cells appeared obvious CPE 3 days after 100 TCID50 virus was (Fig. 6A) inoculated. Pre-incubation of the virus with N protein specific antibody could not inhibit the virus infection (Fig.6B), while pre-incubation of the virus with S protein specific antibody Sfab58IgG (Fig. 6C) and the M specific antibody SFab50IgG (Fig.6D), SARS-CoV infection was completely neutralized, the morphology of infected cells remained normal as shown in Figs. 6C and 6D.

FIG. 6. Neutralization of SARS-CoV infection on Vero cells. The CPE resulted from SARS-CoV infection (Left-up) was completely neutralized with 0.5 μg of purified S specific human IgG antibody SFab58IgG (Left-down) and M specific SFab50IgG (Right-down). The N specific IgG antibody SFab20IgG was not able to neutralize the virus infection (Right-up).

TABLE 1

Neutralization of SARS Coronavirus SCV8 By SRAR Patients-derived Human Recombinant IgG Antibodies

Antibodies	Neutralization Titer	
	100%	50%-80%
	(μg)	(μg)
SFab15IgG	0.5	0.12
SFab58IgG	0.25	0.06
SFab59IgG	0.3	0.08
SFab63IgG	N	N
SFab50IgG	0.5	0.25
SFab20IgG	N	N

Note. N: Non-neutralizing at the concentration of more than 5 μg of IgG antibody.

DISCUSSION

The worldwide outbreak of SARS in spring of 2003 raised a very urgent question that is how do we treat the emergent and life-threatening new SARS-CoV infection? Unfortunately, there are no approved antiviral drugs that are highly effective on coronaviruses. Generally, antibodies can recognize and eliminate foreign pathogens by targeting surface molecules of the pathogenic proteins. Therefore, human-derived antibodies provide new therapeutic opportunities to viral diseases. Phage display and recombinant antibody platform technologies have been successfully used to generate human-derived antibodies to a wide range of viral pathogens, and their advantages and applications have been reviewed elsewhere[4-5].

By using phage display technology, we have

isolated a panel of human Fab antibodies to SARS-CoV virus spike (S), membrane (M) and nucleacapsid (N) proteins from blood lymphocytes of 6 HFRS patients in 25-30 day convalesce phase. However, none of the antibodies reacts with E protein. Sequence analysis showed that CDR regions of the antibodies were different in the antibodies to S, M and N proteins, and each was located in an independent phylogenetic branch of antibody family, indicating that the diversities of antibody responses to SARS-CoV invasion in SARS patients, and different antibodies may play a different role in the disease progress.

We are particularly interested in the neutralizing antibodies to SARS-CoV S and M proteins. The spike protein is a large, type I membrane glycoprotein containing distinct functional domains near the amino (S1) and carboxy (S2) termini. The S protein of coronaviruses serves as a dominant neutralization antigen on the virus and is responsive for viral pathogenesis and virulence[11]. It was reported that SARS-CoV can specifically bind to its receptor angiotensinconverting enzyme 2 (ACE2) on host cells and induce the membrane fusion[12]. Human neutralizing antibodies to S domain of SARS-CoV can bind to the receptor and induce cell membrane fusion[13]. M protein is a virus matrix glycoprotein, part of the M protein domain with transmembrane helices is exposed on the surface of the viral particles as part of viral envelop protein and plays an important role in virus assembly. Association of S and M proteins is an essential step in formation of the viral envelope and accumulation of both proteins at the site of virus assembly[14] . Our data show that S and M protein-targeted antibodies isolated from SARS patients can efficiently neutralize SARS-CoV infection. The epitope location bound to the described neutralizing antibodies remains to be identified. Unlike the N specific antibody which could recognize a liner epitope, S or M protein-targeted antibodies obtained in this study are not able to recognize SDS-PAGE-denatured viral proteins, suggesting that the antibodies may recognize conformational epitopes but not the liner epitope. These conformational epitopes may be involved in the binding of virions to cellular receptors and the fusion activity of the virus. The S proteins of some coronaviruses, including MHV and group III coronavirus infectious bronchitis virus, are cleaved into two subunits (S1 and S2) by a cellular protease in virus-producing cells[14-16]. In this study, the 180 kd resulting band precipitated by the SARS-CoV S protein specific antibodies indicated that the SARS-CoV S protein was not cleaved in virus-producing cells.

In addition to the antibody neutralizing activities, we noticed that intra-cellular distributions of the SARS-CoV viral proteins were stained with S-targeted and N-targeted antibodies as shown in Figs. 4A and 4B. A putative nuclear location signal has been found in SARS-CoV N protein[17]. However, our data suggest that it is predominantly cytoplasmic. On the other hand, the intracellular locations of S and M proteins defined by SARS patients-derived antibodies showed a typical endoplasmic reticulum (ER) pathway patten. As a general rule of the coronavirus assembly, SARS-CoV may synthesize the N protein in cytoplasm which interacts with virus RNA to form RNA-nucleaocapsid protein (RNP), while the S and M proteins are synthesized on endoplasmic reticulum (ER) membrane. RNP is associated with M protein on ER and further associated with S protein to form initial virus particles. The virus particles then migrate through the Golgi complex and release the virus out of the cells probably by exocytosis to form mature virus particles.

In summary, we have successfully generated neutralizing IgG antibodies to SARS-CoV S and M proteins from SARS patients-derived antibody library. The S and M proteins are localized in ER and the neutralization determinants on the surface of SARS-CoV are mostly conformational. Control of SARS can be achieved by vaccination. However, development of SARS-CoV vaccine needs more investigations. A possible approach for prevention of SARS in people at high risk of exposure such as health care workers, is to administrate of neutralizing antibodies against SARS-CoV. As previously reported, since SARS patients remain viremic during the acute phase of SARS-CoV infection, intervention with therapeutic neutralizing human recombinant antibodies might prove useful for shortening the viremic period of SARS and for post exposure prophylaxis and treatment of SARS infection. The neutralizing antibodies we generated in this study may be more promising candidates for preventive and therapeutic purpose, and are more helpful for SARS vaccine design.

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