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Development of a Western Blot Assay for Detection of Antibodies against Coronavirus Causing Severe Acute Respiratory Syndrome

Qigai He,[1] Kooi Hoong Chong,[1] Hiok Hee Chng,[2] Bernard Leung,[2] Ai Ee Ling,[3] Ting Wei,[1] Shzu-Wei Chan,[1] Eng Eong Ooi,[4] and Jimmy Kwang[1]*

Animal Health Biotechnology, Temasek Life Science Laboratory, National University of Singapore,[1] Department of Rheumatology, Allergy and Immunology, Tan Tock Seng Hospital,[2] Virology Section, Department of Pathology, Singapore General Hospital,[3] and Environment Health Institute, National Environment Agency,[4] Singapore, Republic of Singapore

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To identify a major antigenic determinant for use in the development of a rapid serological diagnostic test for severe acute respiratory syndrome (SARS) coronavirus infection and to study the immune response during SARS coronavirus infection in humans, we cloned the full length and six truncated fragments of the nucleocapsid gene, expressed them, and purified them as glutathione *S*-transferase-tagged recombinant proteins. The reactivities of the recombinant proteins to a panel of antibodies containing 33 SARS coronavirus-positive sera and 66 negative sera and to antibodies against other animal coronaviruses were screened. A truncated 195-amino-acid fragment from the C terminus of the nucleocapsid protein (N195) was identified that had a strong ability to detect antibodies against SARS coronavirus. No cross-reaction was found between the N195 protein and antibodies against chicken, pig, and canine coronaviruses. The N195 protein was used to develop a Western blot assay to detect antibodies against SARS coronavirus in 274 clinically blinded samples. The specificity and sensitivity of this test were 98.3 and 90.9%, respectively. The correlation between our Western blotting assay and an immunofluorescence assay (IFA) was also analyzed. The results of our Western blot assay and IFA for the detection of SARS coronavirus-positive sera were the same. Thus, the N195 protein was identified as a suitable protein to be used as an antigen in Western blot and other possible assays for the detection of SARS coronavirus infection.

Severe acute respiratory syndrome (SARS) is a newly emerging human disease, first identified in the Guangdong province of China in November 2002. It spread worldwide, affecting 8,360 individuals and resulting in 764 deaths by 31 May 2003 (9). Several laboratories responded quickly by isolating a novel coronavirus (3, 5, 10, 12). Phylogenetic analyses indicated that the SARS coronavirus is not closely related to any of the previously characterized coronaviruses and forms a distinct group within the genus (14).

Coronaviruses are the largest enveloped positive-stranded RNA viruses, with genome sizes ranging from 27 to 30 kb (8, 13). The genomic organization of the SARS coronavirus is typical of coronaviruses, with a characteristic gene order (replicase, spike [S], envelope [E], membrane [M], and nucleocapsid [N]). The structural proteins (S, E, M, and N) function during host cell entry and virion morphogenesis and release.

During virion assembly of the coronavirus, N binds to a defined packaging signal on the viral RNA, leading to the formation of a helical nucleocapsid. N also has a novel nuclear function, which could play a role in pathogenesis. Based on previous findings, N was identified as the target gene for the development of a PCR for diagnosis (4, 6, 18). The N protein of coronaviruses (such as infectious bronchitis virus [IBV]) is highly conserved in each group, is immunogenic, and is abundantly expressed during infection. It has been identified as a suitable candidate for diagnostic applications for animal coronaviruses.

Rapid viral diagnosis will become increasingly critical, both for the control of epidemics and for the management of patients with SARS coronavirus infections. Currently, an immunofluorescence assay (IFA) is the "gold standard" for the detection of SARS coronavirus infection. However, it requires laboratories with biosafety level 3 (BSL-3) facilities, special equipment, and well-trained technicians. Thus, a reliable recombinant protein-based Western blot assay for the detection of antibodies against the SARS coronavirus that is not dependent on culturing of the SARS virus would be useful. It would also decrease the risk of laboratory infections with the live virus. Presumably, this recombinant protein could be made widely available.

In this study, in order to cast light on the possible diagnostic value of the N protein of SARS coronavirus, we expressed and purified full-length N and six truncated N proteins. The reactivities of the recombinant proteins with human SARS coronovirus-positive sera and animal coronavirus-positive sera were tested. The further diagnostic potential of the expressed recombinant protein was revealed by immunoblotting.

MATERIALS AND METHODS

Virus strain. The SARS coronavirus used for this study was isolated and provided by the Singapore General Hospital (isolate SIN2774; GenBank accession number AY283798).

Human sera. The human sera used for this study were collected from various institutions, as listed in Table 1. The convalescent-phase sera were collected from SARS patients who fully recovered and were discharged from the hospital, while the confirmed SARS sera were collected from SARS patients who were still in

* Corresponding author. Mailing address: Animal Health Biotechnology, Temasek Life Science Laboratory, 1 Research Link, National University of Singapore, Singapore 117604, Republic of Singapore. Phone: 65-68727473. Fax: 65-68727007. E-mail: kwang@tll.org.sg.

TABLE 1. Nature and source of sera used in immunoblot assays

Serum group	No. of samples	Origin(s) of serum samples
Convalescent-phase SARS patient sera[a]	6	National Environment Agency, Singapore; Center for Disease Control, Guangzhou, China
Confirmed SARS patient sera[a]	27	Singapore General Hospital, Singapore; Tan Tock Seng Hospital, Singapore
Normal human sera	66	Singapore General Hospital, Singapore; Tan Tock Seng Hospital, Singapore (volunteer blood donors)
Clinically blinded sera	274	Singapore General Hospital, Singapore; Tan Tock Seng Hospital, Singapore

[a] All patients satisfied the World Health Organization definition of SARS. These serum samples were collected from 4 to 49 days postfever (mean, 18.79 days; median, 14.5 days; standard deviation, 11.95 days; standard error of the mean, 2.26 days).

the hospital. The clinical picture for these patients satisfied the World Health Organization definition of SARS (17). Clinical records for these patients were also available. All human sera were inactivated at 56°C for 30 min.

Animal sera. Four IBV-infected chicken sera and 7 transmissible gastroenteritis virus (TGEV)-infected swine sera, obtained from the Temasek Life Science laboratory, together with 12 canine coronavirus-vaccinated dog sera, obtained from Taiwan, were used to check for cross-reactions. Ten stray dog sera and 10 stray cat sera that were provided by the Agri-Food and Veterinary Authority of Singapore were included in this study as well.

Molecular cloning. The supernatant of the SARS coronavirus cell culture was inactivated before it was used for RNA extraction. The viral RNA was extracted by using Trizol reagents (Gibco, Grand Island, N.Y.) and was reverse transcribed to produce cDNA. Amplification of the full length and six truncated fragments of the nucleocapsid was performed by a standard PCR (94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min). The sequences of primers are referred to by their positions within the nucleocapsid gene, with the note that the 5′ ends of the forward and reverse primers contain *Bam*HI and *Sal*I sites, respectively, to facilitate cloning (Fig. 1a). Homology analyses of the full-length nucleocapsid gene compared to human coronaviruses and other animal coronaviruses were performed by bioinformatic methods.

The amplified products were purified with a PCR purification kit (Qiagen, Hilden, Germany), digested with the enzymes *Bam*HI and *Sal*I, ligated into a pGEX-4T3 vector by use of a Rapid Ligation kit (Roche, Penzberg, Germany), and finally transformed into *Escherichia coli* strain JM105. The positive clones were identified by PCR screening and were further confirmed by enzymatic cutting and sequence analysis.

Protein expression and purification. The transformed bacteria were grown to an optical density at 600 nm of 0.5 to 0.6 in Luria-Bertani medium with ampicillin (final concentration, 100 μg/ml) and were induced with 0.15 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 5 h at 37°C. Cells were pelleted and resuspended in 1× phosphate-buffered saline (PBS). The sonicated lysate was centrifuged at 20,000 × *g* for 10 min.

The expressed recombinant fusion proteins were in two forms, as soluble proteins (N210, N195, N80A, and N71) or insoluble proteins (full length, N170, and N80B). The soluble recombinant proteins were incubated with glutathione-Sepharose 4B beads (Amersham Biosciences, Piscataway, N.J.) and eluted with 10 mM glutathione (Sigma, St. Louis, Mo.) in 50 mM Tris-HCl, pH 8.0. The glutathione *S*-transferase (GST) protein was cleaved with thrombin protease (Amersham Biosciences). Dialysis was performed overnight in 1× PBS at 4°C, followed by the removal of GST by use of glutathione-Sepharose 4B. However, the insoluble proteins, which were dissolved in 6 or 8 M urea, were purified with a protein eluter (Bio-Rad).

Western blotting. Purified proteins were immunoblotted onto nitrocellulose membranes (0.45-μm pore size; Bio-Rad). All sera were screened at a dilution of 1:100, followed by incubation with a peroxidase-conjugated secondary antibody (Dako, Glostrup, Denmark) according to the manufacturer's instructions. DAB (3,3′-diaminobenzidine tetrahydrochloride) (Pierce, Rockford, Ill.) was used as the horseradish peroxidase substrate for membrane color development.

IFA. IFAs were performed in laminar-flow safety cabinets in a BSL-3 laboratory. The SARS coronavirus was propagated in Vero E6 cells at 37°C until cytopathogenic effects were seen in 75% of the cell monolayer, after which the cells were harvested, spotted onto Teflon-coated slides, and fixed with 80% cold acetone. Uninfected Vero E6 cells were used as controls for this experiment. Serum samples were tested at a 1:10 dilution and washed with 1× PBS after being incubated either for 90 min, followed by incubation with a fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulin M (IgM), or for 30 min, followed by incubation with a fluorescein isothiocyanate-conjugated anti-human IgG, and were incubated further at 37°C. The slides were subjected to another washing cycle before being monitored for specific fluorescence under an immunofluorescence microscope.

Calculations. The sensitivity and specificity of the Western blot assay were calculated by using the following equations: sensitivity = number of true positive samples/(number of true positive samples + number of false negative samples) × 100 and specificity = number of true negative samples/(number of true negative samples + number of false positive samples) × 100.

RESULTS

Cloning and expression of recombinant proteins. The full length and six truncated fragments of the nucleocapsid gene were amplified with corresponding primer pairs. PCR products of the expected sizes are shown in Fig. 1a. The size of the full-length nucleocapsid gene is 1,269 bp, and the sizes of the six truncated fragments are 630, 585, 510, 213, 240, and 240 bp. The full-length gene and the six truncated fragments were sequenced, and all sequences matched those for the nucleocapsid gene of the SARS coronavirus.

The purified PCR products were ligated into the pGEX-4T3 vector and transformed into *E. coli* JM105 cells. The recombinant plasmids were sequenced and they were all in frame. The products for the expressed GST fusion proteins with expected sizes are shown in Fig. 1b. For the elimination of cross-reactions in human serum that might result from the GST tag, GST was removed by use of thrombin protease from the purified recombinant proteins.

Characterization of recombinant proteins. A Western blot assay was developed to examine the relative pattern of the truncated proteins with the panel antibodies, 33 SARS coronavirus-positive sera and 66 negative sera. From the screening of six truncated proteins, the N210 and N195 proteins were found to be immunodominant and were potential candidates for the detection of SARS coronavirus antibodies. Both were able to detect all 33 SARS coronavirus-positive sera and had the same IgG detection rate, but they had different IgM detection rates. The N195 protein was found to have a high IgM detection rate (15 of 33) compared to N210 (3 of 33) (Table 2). This indicated that the N195 protein is a better candidate for the early detection of SARS coronavirus infection (Fig. 2).

We also found no cross-reaction between the N195 protein and sera against various animal coronaviruses, namely IBV, TGEV, and canine coronavirus. This further revealed that the use of N195 for the detection of human SARS coronavirus antibodies was highly specific and unique.

Development of Western blot assay for SARS. Because of the result described above, the N195 protein was used to develop a Western blot assay, and the detection protocol was optimized. A total of 274 clinical sera which were collected from patients suffering from "probable" and "suspected" SARS, dengue fever, autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, and Sjogren's syn-

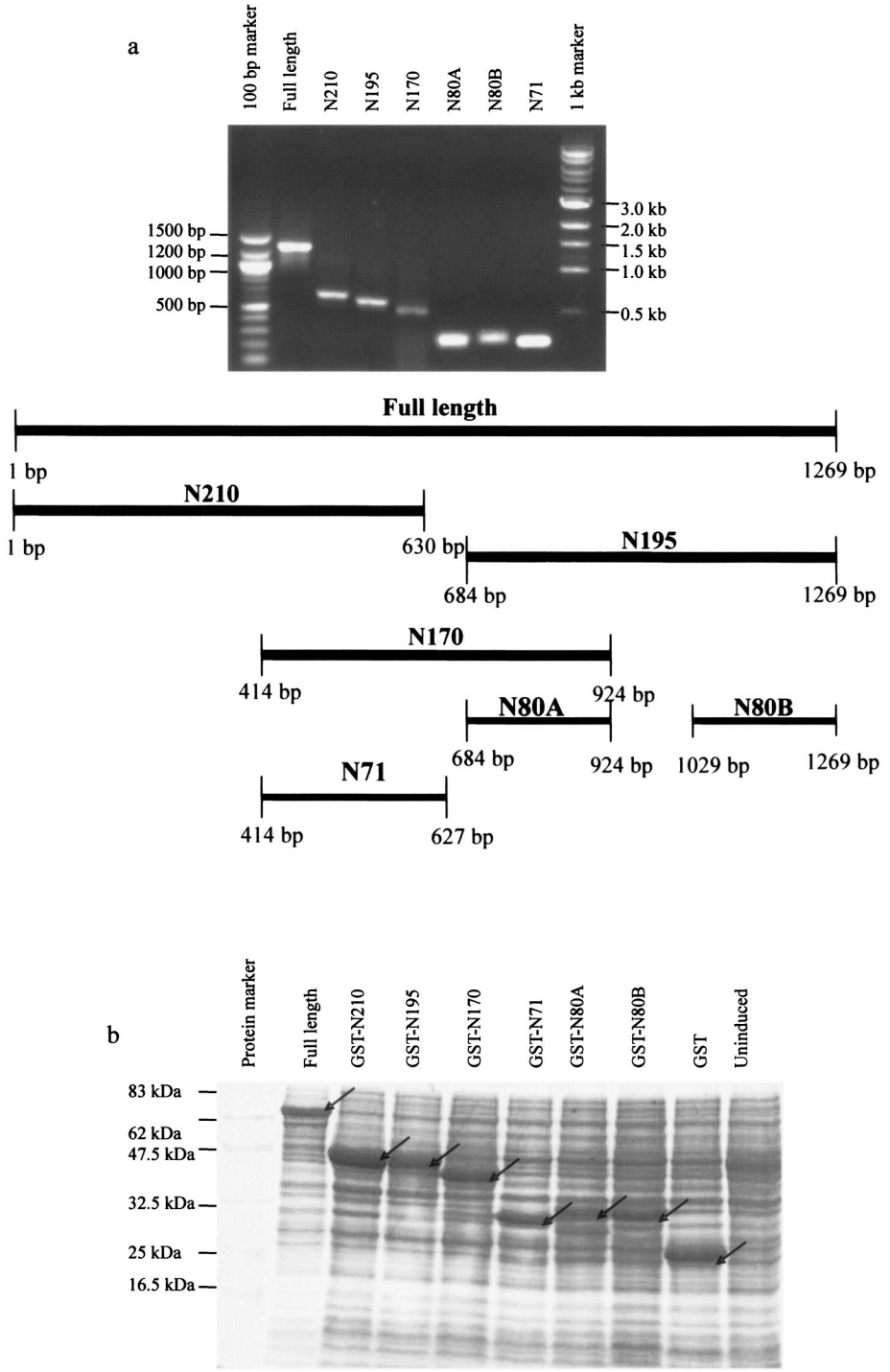

FIG. 1. Purified and expressed fragments of the nucleocapsid gene. (a) Amplified PCR products of the nucleocapsid gene and the relative locations of the truncated fragments. The full length and the truncated fragments of the nucleocapsid were amplified by PCR. N210, bp 1 to 630; N195, bp 684 to 1269; N170, bp 414 to 924 bp; N71, bp 414 to 627; N80A, bp 684 to 924; N80B, bp 1029 to 1269. Samples were loaded in a 1% agarose gel. (b) Expression of GST-fused full-length and truncated fragments of nucleocapsid protein in *E. coli*. Samples were loaded in a sodium dodecyl sulfate–12% polyacrylamide gel. Arrows indicate the positions of the proteins.

drome), aspiration- and community-acquired pneumonia, renal failure, or other diseases were included in a blinded test. These clinical samples also included multiply tested and patient time course samples. The samples were used in a blinded manner to test the accuracy and repeatability of our Western blot assay. With this blinded test, 40 of 44 clinical SARS samples were positive (Table 3). The detection rate was 88.6% (39 of 44) for IgG antibodies and 56.8% (25 of 44) for IgM antibod-

TABLE 2. Detection patterns of the N210 and N195 proteins

Detected antibody	Sera	No. of samples with antibody/total	
		N210	N195
IgG	SARS positive	33/33	33/33
	SARS negative	0/66	0/66
IgM	SARS positive	3/33	15/33
	SARS negative	0/66	0/66

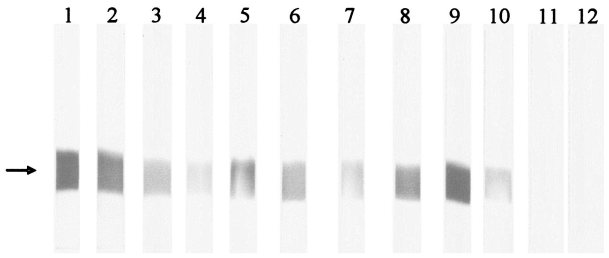

FIG. 2. IgG detection of 10 representative positive samples and 2 representative negative samples. The purified N195 protein was immunoblotted onto a nitrocellulose membrane. Inactivated human antisera were used as the primary antibody at a 1:100 dilution, followed by a peroxidase-conjugated IgG secondary antibody. DAB was used as the horseradish peroxidase substrate, and the membrane was developed for 30 s. The arrow indicates the location of the N195 protein.

ies (data not shown). The detection rate for IgM antibodies increased the total number of positive samples in the overall detection rate for SARS infection by our Western blot assay to 90.9%. With these 40 SARS-positive samples, collected in the range of 4 to 76 days after the onset of fever, the detection rate for IgG antibodies was higher than that for IgM antibodies because of the late serum collection after the onset of fever. No correlation was found between IgG and IgM titers (Table 4) in the antibody fluctuation of IgG and IgM or in day-to-day variability of antibodies.

After screening of the 274 clinically blinded samples, our Western blot test results were found to be highly concordant with the clinical diagnoses. There were a few interesting discoveries, as follows. (i) A set of samples was used to study the time point of SARS antibody detection (Table 4). Three consecutive samples were collected at three different time points (7, 15, and 23 days after the onset of symptoms) from the same patients (patients 15, 16, and 17). SARS antibody detection was negative at 7 days postonset but was positive at 15 and 23 days postonset. (ii) A set of samples was used to study the repeatability of our Western blot assay. The set consisted of three samples from the same patients (patients 22 and 23), all of which had similar results. This further indicated the specificity of the Western blot assay. (iii) Samples from patients who had fever symptoms but did not fulfill the criteria for SARS at the time when SARS was epidemic in Singapore were also included in this study. All of these samples tested negative for SARS coronavirus IgM and IgG antibodies by our Western blot assay. These patients had clinical diagnoses of dengue fever, aspiration- and community-acquired pneumonia, urinary tract infection, and unknown viral fever. (iv) One hundred samples from patients suffering from autoimmune diseases (systemic lupus erythematosus, connective tissue diseases, and inflammatory arthritis) were studied, and four of them showed nonspecific reactions in our Western blot assay.

Since IFA was used as the gold standard for SARS serology detection, we selected 39 representative samples which had been screened by Western blotting for comparison with IFA results to further validate the reliability of our Western blot assay. These included 20 Western blot-positive samples from SARS patients, 5 Western blot-negative but SARS-suspected samples (from 4 to 17 days postfever), and 14 samples from patients with other diseases (Table 4). For this study, 20 samples were positive and 10 samples were negative by both IFA and Western blotting. Samples from patients 18 and 20 demonstrated nonspecific reactions for Western blotting only, while samples from patients 24, 25, 26, and 27 gave positive or nonspecific results for the IFA test only. Two samples (from patients 34 and 35) showed nonspecific binding in both Western blots and IFA. Based on the results for these 39 samples, we found that the overall detection rates of SARS coronavirus antibodies by Western blotting and IFA were the same.

DISCUSSION

The outbreak of SARS has had a major impact on the economies of Asian countries that were affected. At present, many assays have been developed for the diagnosis of SARS coronavirus infection in humans. These assays are based on pathogen confirmation, such as virus isolation; viral sequence

TABLE 3. Summary of the overall detection rate by Western blotting with N195

Serum group	No. of patients	Description of sera	Test result	% Specificity (% sensitivity)
SARS negative	66		All negative	100
SARS positive	33	6 sera from convalescent patients (30 to 45 days postfever) and 27 confirmed SARS patient sera (13 to 38 days postfever)	All positive	(100)
Clinically blinded samples	274	Samples from SARS patients (44 patients) (4 to 76 days postfever), autoimmune disease patients (100 patients), dengue fever patients (12 patients), aspiration- and community-acquired pneumonia patients (12 patients), renal failure patients (12 patients), and patients with no other diseases (94 patients)	40 of 44 confirmed SARS patients sera were positive[a]	98.3 (90.9)

[a] Four of the 100 autoimmune disease sera showed nonspecific reactions in our Western blot assay.

identification, such as by reverse transcription-PCR and real-time PCR; and antibody detection, such as by IFA and whole virus-based enzyme-linked immunosorbent assay (ELISA). However, most of these techniques, such as IFA, reverse transcription-PCR, and real-time PCR, need special equipment and well-trained technicians. The virus from tissue cultures must be operated in a BSL-3 laboratory, emphasizing that further efforts are needed to explore a more convenient, economical, and low-risk procedure. Now, although the SARS outbreak has ceased, the volume of tests for SARS is likely to remain a significant portion of diagnostic laboratories' workloads, especially during the winter months in the northern hemisphere. A test that does not rely on culturing of the SARS coronavirus would thus greatly decrease concerns about laboratory-acquired infections.

The sequence of the nucleocapsid gene of SARS coronavirus was found to have 26 to 32% homology with nucleocapsid genes of various animal coronaviruses. To eliminate possible cross-reactions between the nucleocapsid protein of the SARS coronavirus and nucleocapsid proteins of various animal coronaviruses, we sought a minimal sequence derived from the region encoding the nucleocapsid protein which can detect all infected patients effectively. Several truncated nucleocapsid proteins were expressed by use of an *E. coli* expression system. A truncated nucleocapsid protein of the SARS coronavirus, named the N195 protein in this study, that can effectively detect human antibodies against the SARS coronavirus was identified.

Most foreign polypeptides expressed as fusion proteins at the C terminus of GST can remain soluble and be purified rapidly. However, it was reported that GST could cause cross-reactions with human sera (4). Hence, the GST tag was cleaved from the N195 fusion protein by use of thrombin protease. The purified N195 protein was able to detect all of the SARS coronavirus-positive sera (from 4 to 49 days postfever), including 28 serum samples from Singapore and 5 convalescent-phase serum samples from Guangdong, China. All sera from both regions showed strong reactivities to the N195 protein, derived from the C terminus of the nucleocapsid protein of a Singaporean isolate, similar to previous reports for other coronaviruses (1, 2, 15, 16). Experiments also showed that the N195 protein did not cross-react with antibodies against IBV, TGEV, and canine coronavirus. All of these features indicate that N195 is an ideal protein for SARS antibody detection.

For further investigation of the sensitivity of the N195 protein toward SARS antibodies, a Western blot assay using N195 was developed to screen 274 clinically blinded samples. We were able to identify 40 samples as SARS positive, but later hospital records showed that 44 SARS cases were included in the clinically blinded samples, resulting in 90.9% sensitivity and 98.3% specificity for our Western blot assay.

Based on these studies, the ability of our Western blot assay to distinguish patients with fevers caused by other diseases, such as dengue fever, renal failure, mental disorders, and pneumonia-related diseases, from SARS patients can reduce the number of false-positive diagnoses.

However, antibodies against the truncated protein N195 could not be detected in all patients at an early stage of SARS coronavirus infection. The probable reasons for this inability to detect antibodies in clinically confirmed SARS sera collected at an early stage of the disease are as follows. (i) The nature of the assay may have caused this inability. After SARS infection, no humoral response to the SARS coronavirus is detectable for several days. This is known as the lag period. This was reported by Li et al. (7), who showed that 20 SARS coronavirus-infected patients tested negative for IgM and IgG at week 1 after the onset of symptoms. In order to obtain a higher detection rate for the early stage of infection, we need to develop a more sensitive detection method, such as an ELISA, to shorten the lag period, but this would be unlikely for the first 4 to 5 days of illness. (ii) Individual biological variations in the immune system may be the reason. Some samples collected on day 12 postfever, such as those for patient 22 (Table 4), who was clinically diagnosed as having SARS, were negative by Western blotting and IFA. The reason for this lack of correlation with the clinical diagnosis is not understood at this stage. Some

TABLE 4. Comparison of Western blotting and IFA with 39 selected samples

Sample no. for this study	Patient record no.	Clinical SARS status	Days of fever	Western blot detection[a]		IFA detection[a]	
				IgG	IgM	IgG	IgM
1	1-SS4	+	Unknown	++++	−	+++	−
2	1-SS10[d]	−		−	−	−	−
3	1-SS13[d]	−		−	−	−	−
4	1-SS16[d]	−		−	−	−	−
5	1-SS18[d]	−		−	−	−	−
6	1-SS19[d]	−		−	−	−	−
7	2-SS46[d]	−		−	−	−	−
8	2-SS59	+	26	+++	−	+++	+
9	2-71	+	8	−	+	−	+
10	3-S10	+	4	−	−	−	−
11	3-S17	+	4	+	++	+++	+
12	3-S24	+	74	++++	−	+++	+
13	3-S20	+	49	++++	++	+++	+
14	3-S38	+	76	++	++++	−	−
15[b]	3-S40	+	7	−	−	−	−
16[b]	3-S41	+	15	+	++	+++	++
17[b]	3-S42	+	23	+	+++	+++	++
18	5-1[c]	−		NSR	−	−	−
19	5-4	+	Unknown	+++	++	−	+
20	5-25[c]	−		NSR	−	−	−
21	5-28	+	Unknown	−	++++	−	+
22	5-32	+	12	−	−	−	−
23	7-7	+	17	−	−	+	−
24	6-2[c]	−		−	−	−	+
25	6-3[c]	−		−	−	−	NSF
26	6-4[c]	−		−	−	−	+
27	6-5[c]	−		−	−	−	NSF
28	7-11	+	14	+	−	+	−
29	7-12	+	13	+++	+	+	−
30	7-13	+	13	++++	+++	++	−
31	7-15	+	7	−	−	−	−
32	7-16	+	Unknown	+	+	−	+
33	7-17	+	13	+++	+	++	−
34	7-21[c]	−		NSR	NSR	NSF	NSF
35	7-24[c]	−		NSR	NSR	NSF	NSF
36	9-1	+	Unknown	+	+	+++	+
37	4299	+	11	++	+++	−	+
38	2604:4209	+	11	+++	+	+++	+
39	1605:4153	+	31	+++	−	+++	−

[a] Numbers of plus signs indicate the relative degrees of positive signals, while minus signs denote a negative result or negative signals. NSR, nonspecific reaction; NSF, nonspecific fluorescence. The overall detection results for Western blotting and IFA were 20 of 25 SARS samples and 20 of 25 SARS samples, respectively.

[b] Samples 15, 16, and 17 were collected consecutively from one patient.

[c] Patient had an autoimmune disease.

[d] Patient had another disease.

patients may have a delayed immunological response and may not seroconvert until up to 6 weeks, for as yet unknown reasons (A. E. Ling, personal communication). It was also reported that the mean time to seroconversion for SARS coronavirus infection is 20 days (11, 17). (iii) It may be that the sensitivities of current serological tests are such that they are unable to detect low titers of SARS coronavirus antibodies. A more sensitive test, such as an ELISA, is urgently needed to verify these samples with low titers of SARS antibodies.

Besides the above results, of 100 autoimmune disease samples screened, four nonspecific reactions (for patients 18, 20, 34, and 35) were shown by our Western blot assay. We compared the variability of our Western blot assay and an IFA with autoimmune disease samples: this set comprised eight samples, including the four samples mentioned above, which were selected randomly to be verified by IFA. Two samples (from patients 24 and 26) were positive, and five samples (patients 20, 25, 27, 34, and 35) showed nonspecific fluorescence by IFA (Table 4). These autoimmune patients had high levels of autoantibodies in their sera. We speculate that these high levels of autoimmune antibodies might have caused nonspecific binding in our Western blot assay and IFA. We also could not discount the possibility that these patients might be infected but are asymptomatic.

The IFA has been considered the gold standard for the detection of SARS coronavirus infection. There was full concordance with neutralization tests when both of these tests were done on patient samples (Ooi Eng Eong, personal communication). By this evaluation, the results obtained from our Western blot tests had good agreement with the IFA test results (Table 4). The overall detection rate for both Western blotting and IFA is the same. Although there is no difference in the detection rates of Western blotting and IFA, the latter is cumbersome and labor-intensive, requiring well-trained technicians who are familiar with IFA staining patterns. In addition, this technique relies on SARS coronavirus culturing for antigen and can thus be done only in laboratories with BSL-3 facilities. The development of the Western blot test described here was an attempt to find a suitable alternative serology test for the serological diagnosis of SARS coronavirus infections. In addition, we are also assessing the use of the N195 protein expressed in insect cells by a recombinant insect virus for the development of a technique that is similar to IFA, but without whole SARS coronavirus culturing in a BSL-3 laboratory, to take advantage of the specificity of IFA without the biohazard risk.

In conclusion, by utilizing recombinant DNA techniques, we have cloned and expressed an immunoreactive SARS viral protein, N195. This recombinant antigen is highly reactive to sera from SARS coronavirus-infected patients, with a high sensitivity and specificity. The Western blot test described here seems to be as sensitive as the conventional IFA technique for the detection of SARS coronavirus infection, proving the value of a serological test using the recombinant antigen. We therefore conclude that the N195-based Western blot assay could become an important and economical alternative serological diagnostic test for the detection of SARS coronavirus infection.

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