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Characterization of monoclonal antibody against SARS coronavirus nucleocapsid antigen and development of an antigen capture ELISA

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Abstract

This report describes the production of several MAbs against N195 protein, a major immunodomain of SARS CoV nucleocapsid protein [He, Q., Chong, K.H., Chang, H.H., Leung, B., Ling, A.E., Wei, T., Chan, S.W., Ooi, E.E., Kwang, J., 2004. Development of a Western blot assay for detection of antibodies against coronavirus causing severe acute respiratory syndrome. Clin. Diagn. Lab. Immunol. 11 (2) 417–422.]. One representative IgG1 monoclonal antibody (MAb), S-A5D5, was selected and characterized. S-A5D5 reacted specifically react with both recombinant and native nucleocapsid protein of SARS CoV. The reactivity of S-A5D5 with purified N195 protein and utilization of the MAb as a detector antibody to develop an antigen capture ELISA was assessed. As little as 37.5 pg of purified N protein and 50 $TCID_{50}$ of SARS CoV could be detected by the antigen capture ELISA. Specific binding of the MAb S-A5D5 to both purified N195 and SARS CoV nucleocapsid antigen was effectively inhibited by human SARS positive serum and guinea pig anti-N195 serum. The N protein in N195-spike recombinant baculovirus-infected Sf-9 cells could also be identified. N protein was detected in 18 IFA IgM-positive serum samples collected from SARS confirmed patients, but not in nine samples collected from SARS recovery patient. No false positive results were given when 60 samples from healthy individuals were tested, and no cross-reaction occurred when infectious bronchitis virus (IBV), chicken coronavirus, was tested. This monoclonal antibody-based antigen capture ELISA is thus a powerful tool for early diagnosis of SARS CoV infection.

Keywords: Severe acute respiratory syndrome; SARS coronavirus; Nucleocapsid; ELISA; Monoclonal antibody

1. Introduction

Severe acute respiratory syndrome coronavirus (SARS CoV) is the causative agent of a new and emerging disease worldwide (Drosten et al., 2003; Marra et al., 2003). The disease was widely prevalent in more than 30 countries with 8460 reported cases and causing 804 deaths in 2002–2003 (WHO, 2003). Thus, the development of diagnostic tests for specific and early detection of SARS CoV will contribute to the risk management of the disease. At present, SARS CoV infection is confirmed by the detection of viral RNA via PCR or RT-PCR (Drosten et al., 2004; Poon et al., 2004), however, this is a technically demanding technique and this is susceptible to cross contamination. The determination of infectious virus in samples can be carried out by inoculating cell cultures, such as Vero cells, with a patient specimen, though this is relatively time consuming. Most serological assays developed so far are based on the detection of specific circulating antibodies (Drosten et al., 2003; He et al., 2004). These assays are highly sensitive and specific for detecting antibodies against SARS CoV. However, a lack of detectable antibodies in SARS CoV infected patients at early stage or throughout the whole disease course has been reported. These cases oc-

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curred in SARS patients who were immuno-compromised or who had chronic conditions, e.g., diabetes mellitus or chronic renal insufficiency and may remain afebrile when acutely ill or possess symptoms attributable to underlying diseases, thus delaying SARS diagnosis (Houng et al., 2004). Therefore, more effort should be directed towards developing a simple and inexpensive assay for the detection of SARS CoV proteins. Such tests could be used for early detection and follow-up of patients during treatment and thus reducing the workload of laboratory personnel.

Antibodies against the nucleocapsid protein are longer lived and occur in greater abundance in SARS patients than antibodies against other viral components such as the spike, membrane and envelope proteins (Chang et al., 2004; Chen et al., 2004; Huang et al., 2004; Kim et al., 2004; Tan et al., 2004; Timani et al., 2004; Zhu et al., 2004). This might be due to the higher expression of nucleocapsid as compared with other viral proteins after SARS CoV infection (Rota et al., 2003). These data indicated that nucleocapsid could play a crucial role in antibody response during infection. In our previous work, a major immunodomain of recombinant SARS CoV nucleocapsid (N195) was identified and used to develop a Western blot for the detection of antibodies against SARS CoV infection, the sensitivity and specificity were 98.5 and 100%, respectively (He et al., 2004). From these results, we hypothesize that monoclonal antibodies against N195 would specifically recognize SARS CoV in immunoassays.

In this study, we produced monoclonal antibodies against N195 protein. The monoclonal antibodies were characterized by SARS CoV-infected Vero cells and nucleocapsid-spike fusion protein-based IFA, Western blot, and N195 protein-based ELISA. The isotype of the promising monoclonal antibody, designated as S-A5D5, was determined and was further applied to develop a specific and sensitive antigen capture ELISA for the detection of SARS CoV. The sensitivity and specificity of this antigen capture ELISA was also assessed.

2. Materials and methods

2.1. Cell lines, medium, and additives

RPMI 1640 medium and fetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA). Hypoxanthine–aminopeterin–thymidine (HAT) supplement used for the propagation of hybridoma was purchased from Sigma (St. Louis, MO). Mice myeloma cell S/P2.0 and *Spodoptera frugiperda* (Sf-9) cells were available from our laboratory. Human cells (THP-1, A459) and Vero cells were obtained from the Institute of Molecular Cell Biology (IMCB), Singapore.

2.2. Virus, sera and detection kit

Inactivated SARS CoV (Sin2774 strain) was provided by Singapore General Hospital. Twenty-seven sera that were available from patients presenting symptoms satisfying the World Health Organization (WHO) definition of SARS and 90 sera were obtained from healthy individuals. Sera were provided by Tock Seng Hospital and were heat inactivated at 60 °C for 1 h before use. The recombinant baculovirus bearing the fusion gene encoding N195 and Sc fragment of the spike protein was constructed as described previously (He et al., 2005). A commercial SARS IFA diagnostic kit (EUROIMMUN, Germany), in which inactivated SARS CoV infected Vero cells was used as antigen, was purchased from Medizinische Labordiagnostika AG, Germany. Three serum samples from infectious bronchitis virus (IBV)-infected chickens and IBV M41 strain were also tested. The experiments involving the use of the inactivated SARS CoV or human SARS inactivated sera were performed in a BSL 2 laboratory.

2.3. Protein preparation

SARS CoV N195 and the N-terminal 210 amino acids of nucleocapsid (N210) protein were expressed and purified as described previously (He et al., 2004). N195 was used for animal immunization as well as the antigen in antibody-detection ELISA and Western blot assay while N210 acted as a heterologous antigen for optimization of antigen capture ELISA.

2.4. Establishment of monoclonal antibody

2.4.1. Immunization of mice

Four 4–6-week-old Balb/C mice were injected subcutaneously with 25 μg of purified N195 protein emulsified with an equal volume of adjuvant (SEPPIC, France) for three times with a 2-week interval. Mice received a final booster injection with 25 μg of antigen in 200 μl PBS to the intraperitoneal cavity 2 days prior to hybridoma fusion.

2.4.2. Generation of monoclonal antibody

Mice were sacrificed and their spleen cells harvested. Mice S/P2.0 myeloma cells were in log-phase growth prior to fusion with spleen cells (Yokoyama, 1999). Hybridoma culture supernatants were screened using ELISA, IFA and Western blot as described previously (He et al., 2004). When the desired clones were identified, they were expanded in 75 cm^2 flasks. One week later, the hybridoma suspension was harvested and cell debris pelleted via centrifugation at 400 g for 10 min, followed by collection of the supernatant and storage at −80 °C.

2.5. Characterizations of the monoclonal antibody

2.5.1. ELISA

Fifty microliters of hybridoma supernatant was incubated in 96-well microplates (NUNC, Denmark) coated with purified nucleocapsid protein, and the bound antibody detected

with a 1:2000 dilution of horseradish peroxidase (HRP) labeled rabbit anti-mouse immunoglobulin (Dako, Denmark). After extensive washing, the plates were incubated with *o*-phenylenediamine dihydrochloride (OPD) (Sigma, USA) for 5 min and the reaction was stopped by addition of 12.5 μl 4 M H_2SO_4. The absorbances at 490 nm were read using a Tecan microplate reader.

2.5.2. SARS CoV infected Vero cells-based IFA

Hybridoma supernatants were diluted 1:10 in sample buffer and mixed thoroughly by vortexing, followed by analysis with a commercial kit (EUROIMMUNO, Germany), in which SARS CoV infected Vero cells were used as the fluorescence antigen.

2.5.3. N195-spike fusion protein-based IFA and Western blot

The two techniques were used to further confirm the reactivity to N protein in accordance with our previous descriptions (He et al., 2004, 2005).

2.5.4. Sandwich ELISA

The reactivity of MAb was evaluated with antigen capture ELISA in which purified N195 was employed as the standard antigen and two-fold serially diluted MAb as the detector antibody. Hybridoma supernatant that did not produce MAb served as a negative control.

2.5.5. Isotyping of MAb

Isotyping was performed using a mouse MAb isotyping kit (Amersham Bioscience, England).

2.6. Preparation of N195 protein specific antisera and IgG extraction

Guinea pigs were immunized with 100 μg of purified N195 protein. Booster injections were administered at 2-week intervals. Ten days later the animals were euthanized for serum preparation. Samples were evaluated for antibody against the N195 by N195-based Western blot. Subsequently, IgG was extracted from the antiserum using Protein A affinity chromatography (Sigma, USA) and the concentration determined using a BCA measurement kit (Sigma, USA). The reactivity of the IgG with N protein was assessed by an antigen capture ELISA, as described below.

2.7. Development of antigen capture ELISA

The 96-well flat bottom microtiter plates (Nunc, Demark) were coated with 292 ng of purified IgG in 50 μl carbonate buffer (73 mM sodium bicarbonate and 30 mM sodium carbonate) per well, incubated at 37 °C for 1 h or at 4 °C overnight. Plates were washed three times with PBS-T and blocked with 50 μl of blocking solution (5% nonfat milk in PBS-T) and incubated at 37 °C for 1 h. After the plates were rinsed with PBS-T for three times, 50 μl of purified N195 protein in PBST containing 1% nonfat milk was added. The plate was washed three times with PBS-T, and 50 μl of MAb was added to each well. The plate was washed again and 50 μl of rabbit anti-human immunoglobulin HRP-conjugated antibodies was added at 1:1000 dilution. After another extensive wash with PBS-T, 50 μl of OPD was added to each well. Plates were incubated at room temperature for 5 min and the absorbance was read at 490 nm.

For ELISA optimization, monoclonal antibody and purified IgG were serially diluted two-fold and were used as detector and capture antibody, respectively, in the new assay. Optimization conditions were determined by comparing the homologous (N195) and heterologous (N210) reaction to achieve the highest specificity and the signal-to-noise ratio for this assay. The signal-to noise ratio was calculated by dividing the absorbance of homologous antigen by that of heterologous antigen.

2.8. Sensitivity test

2.8.1. Detection of purified protein

Purified N195 (71 ng/μl) was 10-fold serially diluted with 1% nonfat milk in PBS-T as diluent and used as tested antigen in the capture ELISA format as described above. The diluent was employed as a blank control.

2.8.2. Detection of SARS CoV

Prior to being tested, inactivated SARS CoV culture ($10^{6.0}$ PFU/ml) was treated with sample lysis solution (0.05% Tween-20, 1% Triton, and 5% nonfat milk in PBS) and incubated at 37 °C for 1 h. After centrifugation at 8000 g for 5 min, the supernatant was assayed. Vero, THP-1 and A459 cells were used as negative controls. The detection limits of both standard protein and SARS CoV were determined according to the cut-off value, which was calculated by the formula ($\bar{X} + 3$S.D.).

2.9. Specificity test

2.9.1. Analyses of cross-reaction

Chicken infectious bronchitis virus (IBV), one of the animal coronaviruses, was propagated in SPF embryos. Debris was removed from the harvested virus supernatant by centrifugation. The titer of virus was determined to be $10^{6.0}$ EID_{50}/0.1 ml. IBV was treated using the same sample lysis buffer and methods as those used for SARS CoV, and assayed in the same way.

2.9.2. Inhibition assay

Twenty five microliters of purified N195, at concentration of 150 and 75 ng/μl, respectively, were mixed with guinea pig anti-N195 serum and a serum from a SARS convalescent patient. Additionally, 5×10^3 and 5×10^2 $TCID_{50}$ of inactivated SARS CoV were incubated with

guinea pig anti-N195 serum. Normal guinea pig and human normal sera were included as a negative serum controls. These contents were subsequently incubated at 37 °C for 1 h, followed by incubation at 37 °C for 1 h before sandwich ELISA analysis, as described above. The percent inhibition of antibody binding was calculated by the following formula: % inhibition = [1.00 − (A_{490} of N195 + positive serum)/(A_{490} of N195 + normal serum)] × 100. The specificity of the blocking was confirmed if the percentage of inhibition was greater than 50.

2.10. Quantitative detection of N protein in recombinant baculovirus infected Sf-9 cells

Detection of SARS CoV protein from human infected cells was mimicked using Sf-9 cells expressing recombinant nucleocapsid, protein according to our previous report (He et al., 2005). Pellets were resuspended in the sample lysis solution, incubated at 37 °C for 1 h, 10-fold serially diluted with 1% nonfat milk in PBS-T before being tested. Non-infected Sf-9 cells were used as a negative control. The detection limit of recombinant N protein in the cell lysates was determined according to the cut-off value ($\bar{X}$ + 3S.D.).

2.11. Qualitative detection of N protein in sera from SARS patients

Thirty serum samples obtained from healthy individuals were used in the test to determine cut-off value for the new capture ELISA. Subsequently, 18 serum samples, which were collected from patients infected with SARS CoV during the SARS outbreak in Singapore in 2003 and were shown to be IgM-positive using inactivated SARS CoV-based IFA, 9 and 60 serum samples available from SARS recovery patients and blood donors, respectively, were tested by the antigen capture ELISA.

3. Results

3.1. Production of monoclonal antibody secreting hybridoma

Fusion of spleen cells from immunized Balb/C mice with S/P 2.0 myeloma cells produced several hybridoma clones secreting MAbs against N195 proteins (data not shown). Positive clones were determined through indirect ELISA as having at least a three-fold higher absorbance than that of the background. The positive clones showed great variation in their ability to secret MAb. The hybridoma cell lines yielding the highest antibody titer, S-A5D5, was selected to produce monoclonal antibody for further analyses and experiments.

3.2. Characterization of the selected monoclonal antibody, S-A5D5

3.2.1. Fluorescent staining pattern and reactivity with N protein

After incubation with MAb and FITC-conjugated antibody and subsequent examination by fluorescence microscopy, positive cytoplasmic immunofluorescent stainings of the authentic virus antigen in infected Vero cells and recombinant N195 expressed in Sf-9 cells were shown (Figs. 1A and 2A), identical to those obtained with guinea pig anti-N195 monospecific antibody (Figs. 1B and 2B) and with human SARS patient serum (Figs. 1C and 2C), while fluorescent staining was not observed in the non-infected Vero cells and Sf-9 cells (Fig. 1D and E, Fig. 2D and E). The specific reactivity of the MAb S-A5D5 with purified N195 protein (Fig. 3A) was identical to that of the human SARS positive serum (Fig. 3B), while no reaction was observed when non-antibody secreting hybridoma was tested (Fig. 3C). The titrations of MAb by ELISA, N195-based Western blot, N195-spike fusion protein based IFA and SARS CoV infected cell-based IFA were 1:10^6, 1:10, 1:160 and 1:10, respectively. Finally, the isotypes of the representative MAb, S-A5D5, was determined as IgG1 class; ELISA reactivity with purified N195 is shown in Fig. 4. With the increase in the dilution factors of MAb, absorbance gradually decreased, while absorbances of MAb at 1:1024 and 1:2048 dilution were nearly equivalent to those of the blank controls. Thus, the reactivity was dose-dependent.

3.3. Purification and reactivity of the extracted IgG

To standardize the sandwich ELISA, IgG was isolated and purified from pooled guinea pig anti-N195 sera whose titer was determined to be 1:8000 by Western blot. The concentration of extracted IgG was determined to be 3.75 μg/μl. SDS-PAGE analysis showed two bands of appropriately 47.5 and 26.5 kDa, representing the heavy and light chain regions (data not shown). As expected, a dose-dependent reactivity of purified IgG occurred (Fig. 4). The absorbances were far greater than those obtained with the same concentration of non-immunized guinea pig serum. The absorbances decreased dramatically when the IgG concentration was 0.73 μg/well, and the reactivity of 92.5 ng of IgG was equal to that of the blank IgG control.

3.4. Development and optimization of the capture ELISA

As a first step in the development of our ELISA, MAb and purified IgG were diluted in coating buffer and was used as capture antibodies to coat the microtiter plates, respectively. However, IgG binding to the hydrophobic and hydrophilic microtiter plates was more efficient than the MAb. Therefore, the ELISA plates were coated with purified IgG and incubated at 4 °C overnight or at 37 °C for 1 h

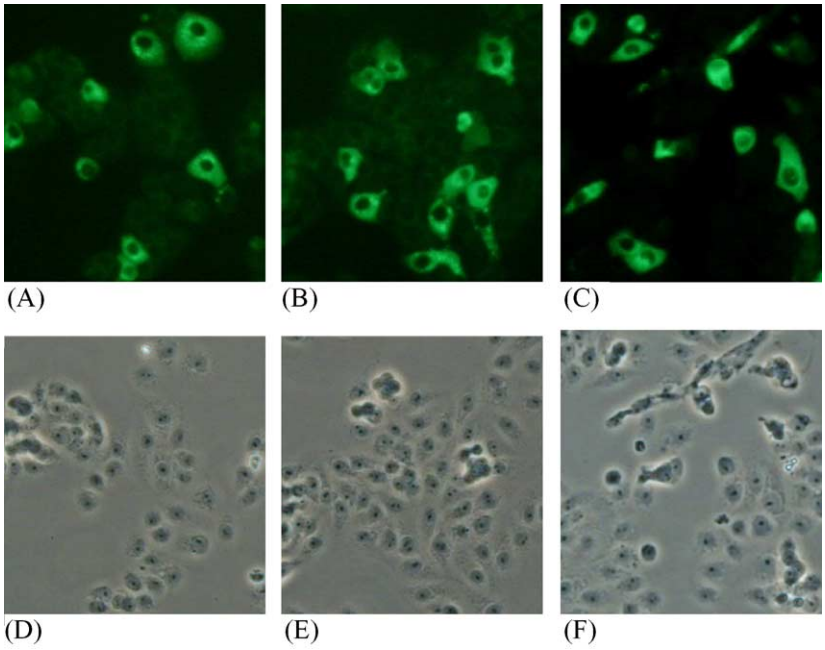

Fig. 1. Fluorescence staining patterns of antibodies in SARS CoV infected Vero cells-based IFA. (A) Monoclonal antibody; (B) polyclonal antibodies; (C) human SARS positive serum; (D–F) side-by-side views of Vero cells staining with MAb, polyclonal antibody and human SARS positive serum under light microscope, respectively.

in the following experiments. When the HRP-conjugated antibody was used at the recommended concentration (1:2000) in the test, 1:80 dilution of capture antibody (2.34 μg/well) and 1:4 dilution of MAb were determined as the optimal working conditions based on the signal-to-noise ratio (16.49) (Table 1). The antigen capture ELISA was standardized using the optimal conditions. The absorbance values of normal Vero, THP-1 and A459 cells were 0.244, 0.25 and 0.259, respectively. Therefore, the cut-off value for detection of viruses in cell culture was determined as 0.267 ($\bar{X}$ + 3S.D.).

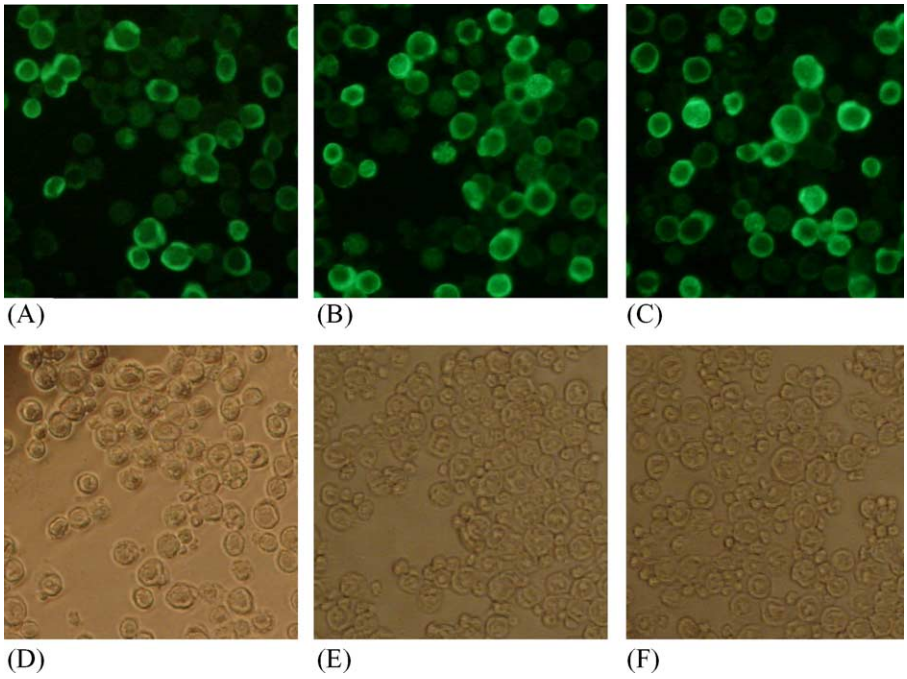

Fig. 2. IFA fluorescence patterns in N195-spike fusion protein based IFA. (A) Monoclonal antibody; (B) polyclonal antibodies; (C) human SARS positive serum; (D–F) side-by-side views of Sf-9 cells staining with MAb, polyclonal antibody and human SARS positive serum under light microscope, respectively.

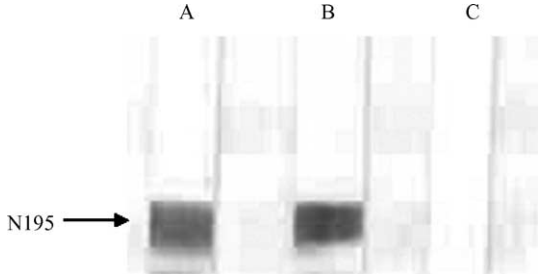

Fig. 3. Western blot identification of MAb against N195 protein. A, monoclonal antibody; B, human SARS positive serum; C, non-antibody secreting cell culture (negative control).

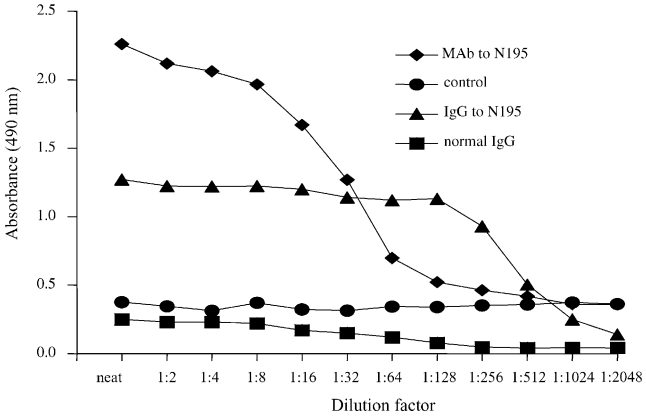

Fig. 4. ELISA reactivity of MAb against N195 protein and isolated IgG. (♦) Different dilutions of MAb; (●) supernatant of non-antibody secreting hybridoma; (▲) various concentrations of anti-N195 IgG; (■) IgG from normal guinea pig serum.

3.5. Minimal detection limit of N protein by the capture ELISA

3.5.1. Purified N protein and native protein in SARS CoV

According to the cut-off threshold (0.267), a 10^{-6} dilution of N195 protein and a 10^{-3} dilution of SARS CoV suspension were considered positive (Fig. 5). So, it was deduced that as little as 37.5 pg of purified N195 protein, 50 $TCID_{50}$ of SARS CoV could be detected.

3.5.2. N antigen from Sf-9 cell lysates

Sf-9 cells (5×10^6 cells/ml) had a 100% infection rate with recombinant baculovirus at 72 h post-infection based on fluorescence assays using MAb against N195 as the primary antibody. A 50 μl aliquot of each dilution of Sf-9 cell lysate supernatant was subjected to ELISA. Absorbance values of 1.376–0.198 were obtained at dilutions of $+10^{-1}$ to 10^{-6}. According to the calculated cut-off value ($0.235 + [3 \times 0.0043] = 0.247$), the test was able to detect a 10^{-4} dilution of infected Sf-9 cells (5×10^2 cells/ml) (Fig. 5). Thus, as few as 25 infected Sf-9 cells could be detected by this ELISA.

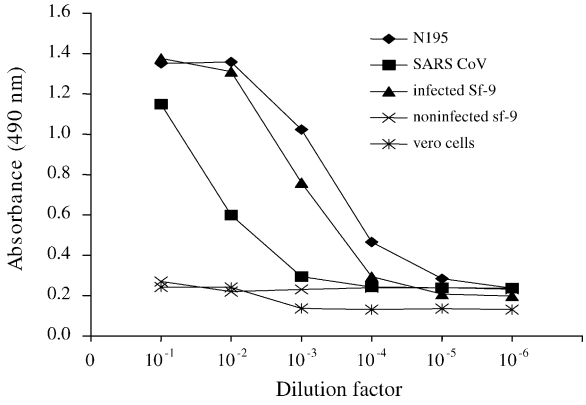

Fig. 5. Detection limit of the antigen capture ELISA. (♦) The purified N195; (■) SARS CoV suspension; (▲) recombinant baculovirus infected Sf-9 cells; (×) non-infected Sf-9 cells; (⋆) non-infected Vero cells.

3.6. Identification of SARS CoV N protein in sera collected at early stage of infection

According to the cut-off value based on the detection of 30 samples from healthy individuals, SARS CoV N protein could be detected in all of the 18 IFA IgM-positive sera, but none of the nine samples from SARS recovery patients using the new ELISA. No false positive results were produced in sera from 60 healthy blood donors.

3.7. Specific binding to N195 antigen is blocked by anti-N195 serum and human SARS positive serum

The recognition site of the MAb on N195 protein was blocked by guinea pig anti-N195 serum and human SARS positive serum, leading to the lower absorbance (Fig. 6). SARS CoV could not be detected after incubation with anti-N195 serum. Human and guinea pig normal serum could not inhibit the binding of MAb to N195 or native viral nucleocap-

Table 1
Checkerboard titration of the working concentrations of the capture and detector antibody

Capture antibody	Detector antibody								
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
1:40	11.48[a]	6.91	6.39	5.99	6.48	5.46	4.54	3.16	2.19
1:80	15.71	16.49	10.54	10.03	1.80	5.18	3.12	1.56	1.73
1:160	2.03	3.23	2.87	2.44	3.51	3.50	3.23	2.60	1.57

In this test, 1:80 and 1:4 dilution of IgG and MAb were used in the standardization of the capture ELISA.

[a] This number represents the signal-to-noise ratio of the sample measured at different combinations of the capture and detector antibody in the antigen capture ELISA.

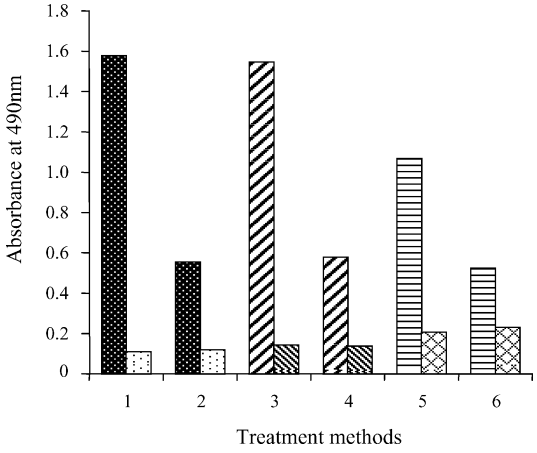

Fig. 6. Inhibition effects of guinea pig anti-N195 serum and human SARS positive serum on the recognition of nucleocapsid antigen by detector antibody. Groups 1 and 3: 3.75 μg of N195; groups 2 and 4: 0.375 μg of N195; groups 5 and 6: 5×10^3 and 5×10^2 $TCID_{50}$ of SARS CoV. The different concentrations of N195 protein or SARS CoV were incubated with guinea pig anti-N195 serum, human SARS positive serum (long bars), guinea pig and human normal serum (short bar), respectively. The contents were subjected to capture ELISA.

sid antigen (longer bars). The calculated inhibition rates were more than 50% (shorter bars). In addition, no cross-reaction was observed when IBV was tested. This result demonstrates that the antigen capture ELISA is highly specific for detecting the nucleocapsid antigen of SARS CoV.

4. Discussion

SARS CoV is an etiological agent causing severe acute respiratory syndrome, a newly emergent disease. There is a global need to develop a sensitive and specific immunoassay to detect SARS CoV. In our previous work, a major immunodomain of N protein (N195) was identified and N195-based Western blot assay was developed to detect antibodies against SARS CoV. Therefore, it is reasonable to assume that a MAb against this N195 protein will improve the test specificity. This paper describes the production of monoclonal antibody against N195 protein as well as the development of an antigen capture ELISA, offering a safe, cost-effective tool for detection of SARS coronavirus.

In this test, the MAb reacted with both N195 and authentic nucleocapsid in SARS CoV, indicating the potential of this MAb to detect SARS CoV by immunoassays. Moreover, the MAb at 1:160 dilution was detectable by Sf-9 cell-based IFA whereas MAb at 1:10 dilution could be detected by SARS CoV infected Vero cells-based IFA. This is partially explained by the fact that the nucleocapsid gene, fused with truncated spike gene, was placed under the transcriptional control of the strong polyhedrin promoter of *Autographa californica* nuclear polyhedrosis virus (AcNPV) in the recombinant baculovirus, leading to an abundance of N195 in infected Sf-9 cells. The recognition of the authentic nucleocapsid antigen indicated that the MAb produced in this work can be used to develop immunoassays, for instance, IFA, for direct detection of SARS CoV from nasopharyngeal aspirates obtained from the patients that are diagnosed as probable or suspected cases under clinical criteria.

Compared to the MAb, IgG displayed stronger binding to microtiter plates and was therefore used as capture antibody with the MAb as detector antibody in this antigen capture ELISA. The detection limit of the test is 37.5 pg of standard protein and 50 $TCID_{50}$ of SARS CoV. This sensitivity is consistent with the previous descriptions of sensitivity in other antigen capture ELISAs. In an attempt to further improve the sensitivity of the assay, MAb against the immunogenic Sc fragment of spike protein (Lu et al., 2004) was produced in this experiment and used as a detector antibody to detect spike protein in infected Sf-9 cell lysates and inactivated SARS CoV. However, this method did not improve sensitivity.

In the specificity test, the specific binding of both recombinant and native nucleocapsid by MAb was uniformly inhibited by guinea pig monospecific antiserum to N195 and human SARS positive serum, but not by human and guinea pig normal sera. Due to a lack of coronavirus infected human samples, IBV was used to assess the specificity of the MAb for other coronaviruses. In this experiment, IBV could not be recognized by the MAb, indicating the MAb's specificity. These observations suggest that the MAb-based assay is specific for detection of SARS CoV infection. Moreover, the N protein was consistently detected in our limited IgM-positive samples but not in eight IgG-positive sera from convalescent patients, indicating the potential of early detection of SARS CoV infection although it needs clinical trials, consistent with new finding (Di et al., 2005). Theoretically, antigen should be released from infected cells or viruses and appear in the host blood system followed by the presence of relative specific antibody. Additionally, due to the abundant expression of N protein compared to other proteins during viral replication in infected tissues, the host immune system is exposed to a larger load of N antigen. Therefore, this antigen capture ELISA, based on MAb to N protein, might provide a more sensitive method for early detection of SARS CoV infection.

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