Cross-Reaction of SARS-CoV Antigen with Autoantibodies in Autoimmune Diseases

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To investigate the significance of the SARS-associated coronavirus (SARS-CoV) antibody, detected by ELISA and indirect immunofluorescence assays (IFA) for the SARS-CoV Vero E6 cell lysates, in non-SARS subjects, 114 serum samples from healthy controls and 104 serum specimens from autoimmune disease patients were collected. The results of ELISA showed that among 114 sera from healthy controls, 4 (3.5%) were positive of SARS-CoV-IgG antibody and 114 (100%) were all negative of SARS-CoV-IgM antibody; the specificity of SARS-CoV-IgG antibody for SARS patients was 96.5%, but the specificity of both SARS-CoV-IgG and -IgM antibodies for SARS patients was 100%. In 58 cases with SLE, positive rates of SARS-CoV-IgG and -IgM antibodies were 32.8% (19/58) and 8.6% (5/58), respectively, in which 11 cases (19%) were positive of both SARS-CoV-IgG and -IgM antibodies; in 10 cases with SS, positive rate of both SARS-CoV-IgG and -IgM antibodies was 10% (1/10); in 16 cases with MCTD, positive rate of SARS-CoV-IgG was 37.5% (6/16), positive rate of both SARS-CoV-IgG and -IgM antibodies was 6.3% (1/16); in 20 cases with RA, one case was positive (5%) of SARS-CoV-IgG. However, of all samples with positive SARS-CoV-IgG and -IgM antibodies for autoimmune diseases and healthy controls, SARS-CoV RNA and antibodies were all negative by RT-PCR and IFA. All sera for negative or positive ELISA results were also negative or positive results using ELISA with Vero E6 cells lysates. These studies showed that SARS-CoV Vero E6 cell lysates for the ELISA to detect SARS-CoV antibodies could lead to the false-positive reactions or cross-reactions of SARS-CoV antibodies in non-SARS diseases and healthy controls, and the false-positive reactions or cross-reactions were related to Vero E6 cell lysates and autoantibodies in non-SARS population. *Cellular & Molecular Immunology*. 2004;1(4): 304-307.

Key Words: SARS-CoV, autoimmune disease, cross-reaction

Introduction

Severe acute respiratory syndrome (SARS) has recently emerged as a new human disease caused by SARS-associated coronavirus (SARS-CoV) (1). So far, the diagnosis of SARS is dependent upon clinical criteria, epidemologic criteria, laboratory criteria and exclusion criteria (2, 3). The identification of the SARS-CoV has led to the development of serologic and virologic tests for the disease. Laboratory diagnostic testings for evidence of SARS-CoV infection include viral isolation, electron microscopy, viral culture, SARS-CoV antibody, or reverse transcription polymerase chain reaction (RT-PCR) using clinical specimens of serum, nasal secretion and stool (1, 4-7). Although laboratory diagnostic tests were very important for cases of SARS-CoV infection, they are still in development and are not available outside research setting (8).

In this report, we detected the serum SARS-CoV antibodies of samples without SARS-CoV infection including 114 healthy controls and 104 patients with systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), rheumatoid arthritis (RA) and mixed connective tissue disease (MCTD) using enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assays (IFA). The results showed that some samples without SARS-CoV infection had positive reaction in the ELISA

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Received for publication Jun 13, 2004. Accepted for publication Aug 16, 2004.

Abbreviations: SARS, severe acute respiratory syndrome; SARS-CoV, SARS-associated coronavirus; RT-PCR, reverse transcription polymerase chain reaction; SLE, systemic lupus erythematosus; SS, Sjogren's syndrome; RA, rheumatoid arthritis; MCTD, mixed connective tissue disease; ELISA, enzyme-linked immunosorbent assay; IFA, indirect immunofluorescence assays; ANA, anti-nuclear antibody; ds-DNA, double strand DNA; ss-DNA, single strand DNA; AHA, anti-histone antibody; AMA, anti-mitochondrion antibody; Scl, sclerosis; RF, rheumatoid factor; CDC, Centers for Disease Control and Prevention.

kit of SARS-CoV antibody detection, but the SARS-CoV RNA and SARS-CoV antibodies were negative by fluorescence quantitative RT-PCR and IFA in these patients.

Materials and Methods

Patients and samples

104 sera from patients with autoimmune diseases and 114 sera from healthy controls were enrolled in this study. 104 patients consist of 58 SLE patients, 10 SS patients, 20 RA patients and 16 MCTD patients. All the peoples are not infected by SARS-CoV. All patients were diagnosed at the Biotherapy Center of Jinan Central Hospital, Clinical Medical College of Shandon University, as well as at the Department of Rheumatology, Shandong People's Hospital. All sera from the patients and healthy controls were drawn from Biotherapy Center of Jinan Central Hospital and were stored at -20 ^{o}C until analysis.

ELISA

SARS-CoV Vero E6 cell lysates used as antigens for the ELISA to detect SARS-CoV antibodies (IgG and IgM) were provided by Huada Genomics Research Center (Beijing, China). The method of ELISA for the detection of SARS-CoV antibodies has been described (9). The Vero E6 cell lysates without SARS-CoV infection used as control antigens were added to the wells in the bottom half of the plate. On the following day, detection of the SARS-CoV antibodies by ELISA coated with Vero E6 cell lysates was done using the same sera.

IFA

IFA was performed by a diluted serum specimen reacted against SARS-CoV-infected Vero E6 cells and uninfected Vero E6 cells. IFA kits of SARS-CoV-infected Vero E6 cells and uninfected Vero E6 cells for SARS-CoV antibodies detection were provided by EuroImmun Medical Laboratory Diganosis Co. Ltd (Germany). Criteria for a positive IFA result included reactivity to infected cells. A sample with an antibody titer of 1:100 is positive. Sera that did not react to infected cells were considered negative (10).

Autoantibody detection

Autoantibodies (ANA, U1RNP, Sm, ds-DNA, ss-DNA, SSA, SSB, AHA, AMA, Scl-70 and RF) were detected by ELISA and IFA using clinical specimens of sera (9). ELISA and IFA kits for autoantibodies detection were provided by EuroImmun Medical Laboratory Diganosis Co., Ltd (Germany).

RT-PCR specific for SARS-CoV

Blood specimens from the autoimmune disease patients with SARS-CoV antibody positive (IgG, IgM or both IgG and IgM) were tested by fluorescence quantitative RT-PCR with specific primers (PF) 5'-CGG CAA AAT GAA AGA GCT CA-3', (PR) 5'-CGC CGT AGG GAA GTG AAG CT-3' and specific probe sequence 5'-CCC AGA TGG TAC TTC TAT TAC CTA-3' (6, 10). All tests were finished at the Central Laboratory of Jinan Central Hospital, Clinical Medical College of Shandong University.

Table 1. *The positive rates of SARS-CoV IgG and IgM antibodies in 218 serum specimens.*

	n	IgG^+ (%)	IgM^+ (%)	IgG^+IgM^+ (%)
healthy	114	4 (3.5%)	0 (0%)	0 (0%)
SS	10	0 (0%)	0 (0%)	1 (10%)
RA	20	1 (5%)	0 (0%)	0 (0%)
MCTD	16	6 (37.5%)	0 (0%)	1 (6.3%)
SLE	58	19 (32.8%)	5 (8.6%)	11 (19%)

Statistical analysis

False-positive rates for autoimmune disease patients without SARS-CoV infection were counted by direct counting. The specificity of SARS-CoV antibody for SARS patients was counted by χ^2 test.

Results

Detection of antibodies to SARS-CoV by ELISA with SARS-CoV Vero E6 cell lysates

The results showed the specificity of SARS-CoV-IgG antibody for SARS patients was 96.5%, but the specificity of both SARS-CoV-IgG and -IgM antibodies for SARS patients was 100%. Of 114 sera from healthy controls, 4 (3.6%) were positive of SARS-CoV-IgG antibody and 114 (100%) were all negative of SARS-CoV-IgM antibody; in 58 cases with SLE, positive rates of SARS-CoV-IgG and -IgM antibodies were 32.8% (19/58) and 8.6% (5/58), respectively, in which 11 cases (19%) were positive of both SARS-CoV-IgG and -IgM antibodies; in 10 cases with SS, positive rate of both SARS-CoV-IgG and -IgM antibodies was 10% (1/10); in 16 cases with MCTD, positive rate of SARS-CoV-IgG was 37.5% (6/16), positive rate of both SARS-CoV-IgG and -IgM antibodies was 6.3% (1/16); in 20 cases with RA, one case was positive (5%) of SARS-CoV- IgG (Table 1).

Detection of antibodies to SARS-CoV by ELISA with Vero E6 cell lysates

All sera from patients with autoimmune diseases and healthy controls for a negative ELISA result were negative using ELISA with Vero E6 cell lysates. Forty-eight serum samples from patients with autoimmune diseases and healthy controls for a positive ELISA result were also positive using ELISA with Vero E6 cell lysates.

Autoantibody

The relationship between SARS-CoV-IgG and -IgM antibodies and autoantibodies in the sera from patients with autoimmune diseases by ELISA with SARS-CoV Vero E6 cell lysates was showed in Table 2 (9). In 4 cases with SARS-CoV-IgG positive of healthy controls, two cases were anti-nuclear antibody (ANA) positive, and other cases were ANA negative. One RA patients with SARS-CoV-IgG antibody positive was also ANA positive. All sera from SLE, MCTD and SS with SARS-CoV-IgG or -IgM

Table 2. *The relationship between SARS-CoV antibodies and autoantibodies in the sera from 26 patients with MCTD and SS.*

No.	Age	Disease	Autoantibodies	SARS-CoV antibodies (IgG/IgM)
1	38	MCTD	SSA(+),ds-DNA(-),Scl-70(+),ANA1:100(+)	-/-
2	42	MCTD	ENA(+),ds-DNA(+),ANA1:1000(+)	+/-
3	72	MCTD	SSA(+),UIRNP/Sm(+),ds-DNA(-),ANA1:320(+)	+/+
4	29	MCTD	SSA(+),UIRNP/Sm(+),ds-DNA(-),ANA1:320(+)	+/-
5	31	MCTD	SSA(+),UIRNP/Sm(+),ds-DNA(-),ANA1:100(+)	+/-
6	44	MCTD	ds-DNA(-),Scl-70(+),ANA1:100(+)	-/-
7	28	MCTD	UIRNP/Sm(+),ds-DNA(-),ANA1:100(+)	-/-
8	18	MCTD	UIRNP/Sm(+),ds-DNA(-),ANA1:100(+)	-/-
9	57	MCTD	UIRNP/Sm(+),ds-DNA(-),ANA1:320(+)	-/-
10	56	MCTD	AMA(+),ds-DNA(-),ANA1:320(+)	-/-
11	50	MCTD	SSA(+),SSB(+),UIRNP/Sm(+),ANA1:1000(+)	+/-
12	40	MCTD	SSB(+),ds-DNA(+),ANA1:100(+)	-/-
13	27	MCTD	UIRNP/Sm(+),ds-DNA(-),ANA1:100(+)	-/-
14	23	MCTD	SSA(+),UIRNP/Sm(+),ds-DNA(-),ANA1:100(+)	+/-
15	27	MCTD	UIRNP/Sm(+),ds-DNA(-),ANA1:100(+)	+/-
16	21	MCTD	ds-DNA(-),Scl-70(+),ANA1:100(+)	-/-
17	32	SS	SSA(+),SSB(+),ds-DNA(-),ANA1:100(+)	-/-
18	37	SS	SSA(+),UIRNP/Sm(+),ds-DNA(-),ANA1:320(+)	-/-
19	42	SS	SSA(+),ds-DNA(-),ANA1:320(+)	-/-
20	68	SS	SSB(+),ds-DNA(-),ANA1:100(+)	-/-
21	66	SS	SSA(+),SSB(+),ds-DNA(-),ANA1:100(+)	-/-
22	55	SS	SSA(+),SSB(+),ds-DNA(-),ANA1:100(+)	+/+
23	21	SS	SSA(+),ds-DNA(-),ANA1:100(+)	-/-
24	15	SS	SSA(+),SSB(+),ds-DNA(-),ANA1:100(+)	-/-
25	40	SS	SSA(+),ds-DNA(-),ANA1:100(+)	-/-
26	24	SS	SSA(+),ds-DNA(-),ANA1:100(+)	-/-

antibody positive were ANA positive and had other autoantibodies.

Detection of antibodies to SARS-CoV by IFA

Four normal serum samples from healthy controls and 44 serum samples from patients with autoimmune diseases were positive for antibodies against SARS-CoV by ELISA with SARS-CoV Vero E6 cell lysates (Table 1), but no positive results were detected in these serum specimens by IFA and the specificity of IFA was 100%.

Detection of viral RNA of SARS-CoV by RT-PCR

To provide the evidence that SARS-CoV antibodies were associated with autoantibodies, specimens from patients of autoimmune disease with SARS-CoV antibody positive were tested by fluorescence quantitative RT-PCR assays. All samples which had positive SARS-CoV-IgG and -IgM antibodies for autoimmune diseases were negative.

Discussion

Serologic testings for SARS-CoV antibody consist of IFA and ELISA that are specific for total IgG, IgM and IgA antibodies produced after infection (4-11). US Centers for Disease Control and Prevention (CDC) have announced that the tests on sera from 384 persons without SARS-CoV infection were all negative and antibodies against other human and nonhuman coronaviruses did not react in these assays (11). Detection of the SARS-CoV-IgG and -IgM antibodies has been done by ELISA with SARS-CoV Vero E6 cell lysates for 114 healthy persons without SARS and all persons were SARS-CoV-IgM negative and four persons (3.5%) were SARS-CoV-IgG positive, two of whom were ANA positive and others were ANA negative. We estimated that the specificity of both SARS-CoV-IgG and -IgM antibodies for SARS patients was 100%. These findings indicated that the serologic methods were specific for detection of antibody against SARS-CoV and had a low false-positive rate (6, 11).

The serum samples from 44 patients with autoimmune diseases were positive for antibodies against SARS-CoV by ELISA with SARS-CoV Vero E6 cell lysates, and 48 serum samples with SARS-CoV antibody positive including 4 healthy controls were negative by IFA and RT-PCR. The specificity of IFA and RT-PCR is extremely good (100%), since no cross-reactions were detected in non-SARS autoimmune disease patients and healthy controls. However, 48 serum samples with SARS-CoV antibody positive were also positive using ELISA only with Vero E6

cell lysates. These results indicate that there were cross-reactons with non-SARS population to detect SARS-CoV antibody by ELISA with SARS-CoV Vero E6 cell lysates (9, 10).

Antibodies to the SARS-CoV were found in serum samples only from patients who were in convalescence period of SARS infection, but not in human serum samples banked before the SARS outbreak. This observation suggested that the SARS-CoV was new to human population (12). In this reports, the ELISA kit coated by SARS-CoV Vero E6 cell lysates were not purified SARS-CoV antigen. Because the serum samples from patients with autoimmune disease had a lot of autoantibodies to cell antigens, and the Vero E6 cell lysates contain many antigens, the autoantibodies in serum from the autoimmune disease can respond to these cell antigens when using the ELISA kit with SARS-CoV Vero E6 cells lysates, so false-positive of SARS-CoV antibody are found (9). The results by IFA and RT-PCR for the patients with false-positive of SARS-CoV antibody were negative. This result suggested that the false-positive reactions were cross-reactions. The false-positive reactions or cross-reactions can be avoided if coated antigen is only purified antigens or recombinant antigens for SARS-CoV (6-10).

So far, SARS patients with autoimmune diseases have not been reported (6). SLE patients may have SARS-like symptoms with fever, low-level white blood cell, respiratory symptoms, and pneumonia. These patients must be excluded as a suspect or probable SARS cases (9). Because some SARS patients do not have detectable SARS-CoV antibodies during the acute phase of their illness, and some SARS patients have detectable SARS-CoV antibodies after 14 days of illness onset by ELISA, a definitive diagnosis of SARS should be made with antibody testing more than 21 days after the onset of initial symptoms (8-11). When the antibody testing was done by ELISA, a suspect or probable SARS patient may be an autoimmune disease patient if laboratory test result for SARS-CoV antibody is positive within 14 days of illness onset. If laboratory test result for SARS-CoV antibody is positive after 14 days of illness onset, SARS-CoV antibody and autoantibodies should be tested in this patient serum sample banked before, and negative SARS-CoV antibody and positive autoantibodies results are reliable enough to rule out SARS-CoV infection.

Newly developed tests, including the rapid RT-PCR for SARS-CoV RNA in secretions and specific SARS-CoV antibodies in serum, will be of great value in defining and differentiating SARS from other diseases.

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