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Longitudinal Analysis of Severe Acute Respiratory Syndrome (SARS) Coronavirus-Specific Antibody in SARS Patients

Shan-Chwen Chang,[1]* Jann-Tay Wang,[1] Li-Min Huang,[2] Yee-Chun Chen,[1] Chi-Tai Fang,[1] Wang-Huei Sheng,[1] Jiun-Ling Wang,[1] Chong-Jen Yu,[1] and Pan-Chyr Yang[1]

Department of Internal Medicine[1] and Department of Pediatrics,[2] National Taiwan University Hospital, Taipei, Taiwan

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The serum antibodies to severe acute respiratory syndrome (SARS) coronavirus of 18 SARS patients were checked at 1 month and every 3 months after disease onset. All of them except one, who missed blood sampling at 1 month, tested positive for the immunoglobulin G (IgG) antibody at 1 month. Fifteen out of 17 tested positive for the IgM antibody at 1 month. The serum IgM antibody of most patients became undetectable within 6 months after the onset of SARS. The IgG antibody of all 17 patients, whose serum was checked 1 year after disease onset, remained positive.

Severe acute respiratory syndrome (SARS) is an emerging infectious disease that caused a global epidemic in 2003 (12). The high mortality rate of the disease and its easy transmission to health care workers characterize its clinical importance (1, 10, 12, 13). The clinical manifestations, laboratory findings, radiologic presentations, and outcomes of SARS for patients have been well described (3, 9, 12). Previous reports also found that the specific antibody to SARS-associated coronavirus (SARS-CoV) appears as early as 9 days after the disease onset and that a high level of antibody could last for 1 to 2 months after the onset of SARS (2, 5, 9). However, studies concerning the long-term evolution of specific antibodies, including immunoglobulin G (IgG) and IgM, to SARS-CoV remain limited (14). This study was conducted at the National Taiwan University Hospital (NTUH) to illuminate the above issue.

During the SARS epidemics in Taiwan in 2003, there were 76 SARS patients with pneumonia identified and treated at NTUH (13). Sixty-one of the 76 patients survived their SARS disease. Among the 61 patients, 18 patients were regularly subjected to follow-up exams at the outpatient clinics at NTUH for more than 1 year after being discharged. The other 43 patients were followed for 3 to 6 months after their discharges. For the 18 patients who were examined for 1 year, SARS was diagnosed based on a positive reverse transcription-PCR result for SARS-CoV on their initial throat swabs and/or the seroconversion of the IgG-specific antibody to SARS-CoV in all patients. The male-to-female ratio of this group was 7:11. Their ages ranged from 24 to 71 years, with a median age of 45.5 years. No children were included in this study. All 18 patients had pneumonic lesions on their chests according to radiographs, and five of them developed respiratory failure during the course of the disease. None of them had any previous underlying disease.

Serum samples used in this study were collected from the 18 SARS patients at 1 month, 3 months, 6 months, 9 months, and 12 months after the onset of their SARS infections. Ten serum samples from healthy volunteers and 10 other serum samples from adult patients with bacteremic pneumonia, collected 17 to 30 days after their disease onsets, were also included in the test for comparison. All of the serum samples were measured for IgM- and IgG-specific antibodies to SARS-CoV using a commercially available indirect immunofluorescent assay (IFA) (Euroimmune, Lübeck, Germany) (2, 4). This test utilizes slides coated with SARS-CoV-infected cells together with noninfected cells to detect specific antibodies in patient serum samples. A reaction with a serum dilution of 1:10 or higher is considered positive (for both IgM and IgG). There is both a negative and a positive control provided by the test kit for each run of the test. The test procedures we used, and our interpretation of the results was according to the manufacturer's instructions. The results were expressed as the reciprocal of the highest dilution of serum that gave a positive fluorescent reaction.

Blood sampling was missed for one SARS patient at 1 month, for three SARS patients at 3 months, for one SARS patient at 6 months, for one SARS patient at 9 months, and for one SARS patient at 12 months after the disease onset. Therefore, there were a total of 83 serum samples from SARS patients. All 20 blood samples from the healthy volunteer and the adult patients with bacteremic pneumonia were negative for both IgM and IgG against SARS-CoV. The titers of the specific antibodies and the initial C-reactive protein (CRP) levels (normal range, <0.8 mg/dl) of the 18 SARS patients, as well as their peak CRP levels during their respective disease courses are described in Table 1. The geometric means ($\log_{10}$) of the IgG titers of the 18 SARS patients are illustrated in Fig. 1.

Of the 18 SARS patients except patient 17, whose serum sample at 1 month after disease onset was unavailable, 15 patients had detectable IgM to SARS-CoV in their sera collected at 1 month after the disease onset. With the exclusion of patient 16, whose serum samples were not collected at 3, 6, and 9 months after the disease onset, IgM antibodies were undetectable in 2 patients at 1 month after the disease onset, in 10 patients at 3 months, in 16 patients at 6 months, and in all 17 patients at 12 months. The peak serum IgG titers in all patients except patient 17 appeared at 1 month or 1 to 3 months after

* Corresponding author. Mailing address: Division of Infectious Diseases, Department of Internal Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei, Taiwan. Phone: 886-2-23123456, ext. 5401. Fax: 886-2-23971412. E-mail: sc4030@ha.mc.ntu.edu.tw.

TABLE 1. CRP levels and serology test results of 18 SARS patients

Patient	Development of respiratory failure	Throat swab RT-PCR result[a]	CRP level (mg/dl)[b]		Serology test result at the indicated period after disease onset[c]									
			Initial	Peak	1 mo		3 mo		6 mo		9 mo		12 mo	
					IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
1	No	−	0.04	1.05	1:32	1:3,200	Missed	Missed	−	1:320	−	1:100	−	1:100
2	No	−	0.2	8.96	1:32	1:3,200	−	1:1,000	−	1:320	−	1:320	−	1:320
3	No	−	0	1.94	1:100	1:320	Missed	Missed	−	1:100	−	1:100	−	1:100
4	No	−	1.32	8.68	1:320	1:3,200	1:10	1:1,000	−	1:320	−	1:320	−	1:320
5	No	+	0	0.46	1:32	1:1,000	1:10	1:320	−	1:320	−	1:320	−	1:320
6	No	−	3.89	5.66	1:32	1:320	−	1:320	−	1:320	−	1:100	−	1:100
7	No	−	1.64	5.46	1:32	1:3,200	1:10	1:1,000	−	1:320	−	1:320	−	1:320
8	Yes	−	4.44	11.6	1:10	1:1,000	−	1:1,000	−	1:320	−	1:320	−	1:320
9	No	−	4.05	4.05	1:320	1:320	1:32	1:320	1:32	1:100	1:10	1:100	−	1:100
10	Yes	+	5.67	12	1:10	1:1,000	−	1:320	−	1:320	−	1:320	−	1:100
11	Yes	+	5.83	12	1:100	1:1,000	−	1:320	−	1:320	−	1:320	−	1:100
12	Yes	−	12	12	1:32	1:3,200	−	1:3,200	−	1:320	−	1:320	−	1:320
13	Yes	−	0.68	5.66	1:32	1:320	−	1:320	−	1:320	−	1:320	−	1:100
14	No	+	6.03	8.0	1:320	1:1,000	1:10	1:320	−	1:320	−	1:320	−	1:320
15	No	−	0.686	0.686	−	1:320	−	1:100	−	1:100	−	1:100	Missed	Missed
16	No	−	3.82	9.43	1:10	1:1,000	Missed	Missed	Missed	Missed	Missed	Missed	−	1:320
17	No	−	2.28	2.82	Missed	Missed	−	1:100	−	1:100	−	1:100	−	1:32
18	No	Missed	3.76	3.76	−	1:1,000	−	1:320	−	1:320	−	1:320	−	1:320

[a] RT, reverse transcription.
[b] CRP normal range, <0.8 mg/dl.
[c] −, negative.

the disease onset. A drop (4.4-fold on average) in IgG titers was evident between 1 month and 6 months after the disease onset. All of the patients except patient 15, whose serum sample was not collected at 12 months after the disease onset, had detectable IgG antibodies in their sera 12 months after the disease onset.

There was no correlation between the IgG titer checked 1 month after disease onset and the patients' ages, initial CRP levels, peak CRP levels, or development of respiratory failure as determined by statistical analysis ($P = 0.43$, 0.57, 0.17, and 0.999, respectively).

Prior studies pointed out that (i) the specific antibody to SARS-CoV appears as early as 9 days after the disease onset and a high level of the antibody can last for 1 to 2 months (3, 9), (ii) IgM and IgG to SARS-CoV appear at almost the same time (4, 6, 14), (iii) the peak IgG titer is usually noted 4 to 12 weeks after the disease onset (7, 14), and (iv) the IgM to SARS-CoV is usually undetectable 180 days after the disease onset (14). Our present study demonstrated similar results. However, since our primary goal in this study was not to determine the actual time when the peak serum antibody titer appeared, we did not collect the serum samples at shorter intervals. Therefore, we could not determine the exact time point when the peak serum antibody titer appeared.

Woo et al. showed that the IgG-specific antibody to SARS-CoV can last for 240 days (14). However, in their study, a tendency toward a continuous drop in the IgG titer was noted until day 240 after disease onset, which raised a question about

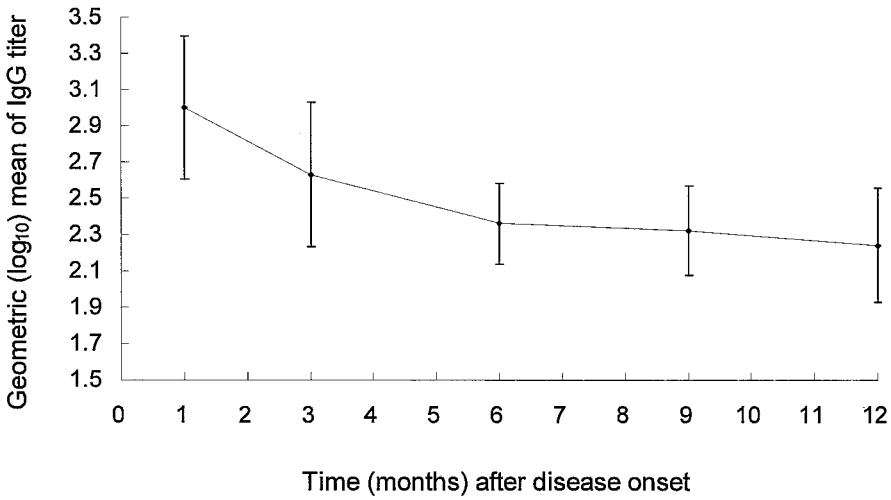

FIG. 1. Geometric means ($\log_{10}$) and standard deviations of IgG titers of 18 SARS patients.

how long the IgG-specific antibody would actually last. Our study demonstrates that the specific IgG antibody can persist up to 1 year after disease onset. In addition, our data also demonstrate that the drop in the titer of IgG-specific antibody is much slower from 6 to 12 months after disease onset than during the earlier period in the disease course. We think that the strength of our present study is that none of the enrolled patients had underlying diseases before contracting SARS-CoV, which means that their immune responses to SARS-CoV infection are therefore assumed to be more representative of patients with normal immune systems. In addition, to our knowledge, this is the study with the longest follow-up period for antibody responses to SARS-CoV as of this writing.

According to our data, IgM to SARS-CoV usually became undetectable 6 months after disease onset, and the titers of IgG became much lower than initial levels (<1:1,000) 6 months after disease onset. Therefore, a positive IgM result and a high titer of IgG (>1:1,000) to SARS-CoV might be useful in determining whether a patient has had a recent SARS-CoV infection. Although 17 out of 18 patients received steroids as one component of their treatment for SARS (9), they still exhibited a positive antibody response to SARS-CoV. Our finding that steroid treatment does not interfere with the antibody response to SARS-CoV after infection had also been demonstrated in one previous study (6).

Our previous study indicated that the initial CRP level was an independent factor in predicting mortality in SARS patients (13). Peiris et al. proposed that the exacerbation of clinical conditions in the second week of the disease course in SARS patients correlated with the appearance of immune responses and assumed that the clinical exacerbation might be due to the uncontrolled immune response (8). However, both the initial and peak CRP levels did not show any significant correlation with the titers of IgG in the present study. This might be because the CRP stood for a nonspecific host response and thus did not necessarily correlate with the host's specific immune response.

Patients who developed respiratory failure during their SARS disease courses did not have significantly higher IgG titers than those who did not develop respiratory failure. Although the number of cases of respiratory failure in the present study was small, this finding might suggest that there was no correlation between the clinical outcome and the specific humoral immune response in SARS patients.

There are some limitations in our present study. First, we did not check the neutralizing antibody to SARS-CoV because of the culture for SARS-CoV is restricted for the sake of safety. This is a weak point of this study for our understanding of the whole humoral response to SARS-CoV infection. However, a recent study by Temperton et al. demonstrated that the neutralizing antibody may last up to 250 days after the disease onset (11). Second, we did not use any antibody test other than IFA, such as an enzyme-linked immunosorbent assay, to determine the antibody response and compare the results with those determined by IFA. However, some previous studies have demonstrated that the antibody responses determined by IFA and enzyme-linked immunosorbent assays were similar (4, 6). Third, only 29.5% of SARS patients who survived and were treated in our hospital were followed for more than 1 year and enrolled in this study. This is because most surviving SARS patients recovered clinically in 1 to 3 months and did not agree to be followed for such a long time. The antibody response to the SARS-CoV infection in our 18 patients thus might not be representative of the whole spectrum of patients, especially those patients with underlying diseases and/or impaired immune systems.

In summary, our work outlined the evolution during a 1-year period of titers of specific antibodies to SARS-CoV in SARS patients who had pneumonia during their disease courses. Our results showed that anti-SARS-CoV IgM might be useful in determining whether a patient has had a recent SARS-CoV infection. We also demonstrated that anti-SARS-CoV IgG may persist for up to 1 year after the illness. However, it is still not known how long the virus-specific serum IgG antibody may persist after infection with SARS-CoV.

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