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Serological Responses in Patients with Severe Acute Respiratory Syndrome Coronavirus Infection and Cross-Reactivity with Human Coronaviruses 229E, OC43, and NL63

K. H. Chan, V. C. C. Cheng, P. C. Y. Woo, S. K. P. Lau, L. L. M. Poon, Y. Guan, W. H. Seto, K. Y. Yuen, and J. S. M. Peiris*

Department of Microbiology, The University of Hong Kong and Queen Mary Hospital, Hong Kong, SAR, People's Republic of China

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The serological response profile of severe acute respiratory syndrome (SARS) coronavirus (CoV) infection was defined by neutralization tests and subclass-specific immunofluorescent (IF) tests using serial sera from 20 patients. SARS CoV total immunoglobulin (Ig) (IgG, IgA, and IgM [IgGAM]) was the first antibody to be detectable. There was no difference in time to seroconversion between the patients who survived ($n = 14$) and those who died ($n = 6$). Although SARS CoV IgM was still detectable by IF tests with 8 of 11 patients at 7 months postinfection, the geometric mean titers dropped from 282 at 1 month postinfection to 19 at 7 months ($P = 0.001$). In contrast, neutralizing antibody and SARS CoV IgGAM and IgG antibody titers remained stable over this period. The SARS CoV antibody response was sometimes associated with an increase in preexisting IF IgG antibody titers for human coronaviruses OC43, 229E, and NL63. There was no change in IF IgG titer for virus capsid antigen from the herpesvirus that was used as an unrelated control, Epstein-Barr virus. In contrast, patients who had OC43 infections, and probably also 229E infections, without prior exposure to SARS CoV had increases of antibodies specific for the infecting virus but not for SARS CoV. There is a need for awareness of cross-reactive antibody responses between coronaviruses when interpreting IF serology.

Severe acute respiratory syndrome (SARS) is a newly emergent infectious disease that posed a major threat to international public health in 2003. A novel coronavirus (CoV) was identified as the etiological agent (4, 7, 12). SARS CoV serology by indirect immunofluorescence (IF) or neutralization tests is regarded as a gold standard for diagnosis of SARS coronavirus infection (12, 13).

In two previous studies, immunoglobulin G (IgG) seroconversion to SARS CoV occurred at a mean of 20 (± 5.1) days (11) and 9 to 18 days (6) after onset of symptoms. Follow-up serum samples from some of these patients collected up to day 60 from onset of symptoms demonstrated persistently high IgG antibody titers (6). In another study, IgM antibody was found to become undetectable by 11 to approximately 24 weeks after onset of illness (15). However, the full serological profile remains largely undefined.

Human coronaviruses 229E and OC43 have long been known to be respiratory pathogens in humans. More recently, NL63 and HKU-1 have been discovered as two novel coronaviruses that can infect humans (17, 19). The 229E and NL63 viruses are group 1 coronaviruses, while OC43 and HKU-1 are classified as group 2 viruses. The taxonomic classification of SARS CoV is still debated, though many argue that it is a distant relative of group 2 coronaviruses (16).

Previous studies had not revealed significant evidence of SARS CoV IF antibody in uninfected healthy controls (2, 7), although most such individuals would be expected to have antibodies to the common human coronaviruses 229E, OC43, and NL63. It has therefore been assumed that a positive SARS CoV IF result can be taken as unequivocal evidence of SARS CoV infection. Conflicting opinions concerning serological cross-reactions between human coronaviruses have remained. When enzyme-linked immunosorbent assays (ELISA) are used, human antibody responses to 229E and OC43 can be clearly distinguished (5, 14). However, some cross-reactive responses have been detected by IF (9), by complement fixation tests (8), and by ELISA using recombinant viral proteins (18). Since additional human coronaviruses have been now discovered, and in view of the global public health importance of diagnosing SARS, it remains important to revisit the question of potential cross-reactions in the human serological responses to coronaviruses.

In the present study, serial sera from 20 SARS patients collected during illness and convalescence up to 6 months postinfection were tested by neutralization tests (NT) and by IF tests for SARS CoV-specific IgG, IgA, IgM, and total antibody (IgG, IgA, and IgM) (IgGAM). Sera from patients with SARS were tested for cross-reaction with other human coronaviruses, 229E, OC43, and NL63, in IF tests. Since HKU-1 still cannot be cultured in vitro, this virus was not included in the serological studies reported here. Furthermore, acute- and convalescent-phase sera from patients with 229E or OC43 infection were tested for cross-reaction with SARS CoV in immunofluorescent and neutralization tests.

MATERIALS AND METHODS

Patients and sera. Six to eight serial serum samples were collected in the first month of illness from a cohort of 20 SARS patients infected at the Amoy

* Corresponding author. Mailing address: Department of Microbiology, the University of Hong Kong, University Pathology Building, Queen Mary Hospital, Pokfulam Rd., Hong Kong, SAR, People's Republic of China. Phone: (852) 2855 4888. Fax: (852) 2855 1241. E-mail: malik@hkucc.hku.hk.

Gardens, Hong Kong, SAR. Eleven of these patients had serum samples collected at 7 months after disease onset. The sera were aliquoted and stored at −80°C until use.

The 20 patients had a mean age of 39.8 years (range, 20 to 65), and the male-to-female ratio was 11:9. All patients presented with high fever, and some had chills, rigors, myalgia, malaise, cough, sore throat, and headache. Fourteen of these patients had diarrhea, with the mean onset at 7.6 ± 1.9 days. Two patients were chronic hepatitis B carriers. In general, these patients had normal hemoglobin (13.6 ± 1.5 g/liter), urea (4.4 ± 1.4 mmol/liter), creatinine (87 ± 16 μM/liter), alanine aminotransferase (42 ± 15 U/liter), aspartate aminotransferase (47 ± 25 U/liter), and white cell count (6.38 ± 2.10 × 10^9/liter) levels. However, they had low lymphocyte count (0.78 ± 0.24 ×10^9/liter), marginally low platelet count (147 ± 33 × 10^9/liter), and elevated creatinine kinase (207 ± 159 U/liter) levels. Seven patients required admission to intensive care, and five had acute respiratory distress syndrome. Six patients had a fatal outcome.

Acute- and convalescent-phase sera from 11 patients with recent OC43 infection and 3 patients with recent 229E infection were retrieved from the serum bank of the Respiratory Pathogen research unit of the Baylor College of Medicine and kindly provided to us by R. B. Couch. These sera had been shown to exhibit significant increases in OC43 and 229E antibody titers when tested by ELISA and microneutralization tests (R. B. Couch, personal communication).

Preparation of CoV-infected smears. SARS-CoV-infected Vero, OC43-infected BSC-1, and 229-infected MRC-5 cells and NL63-infected LLCMK2 cell smears were used for the study. Coronavirus smears were prepared according to a method described previously (2). Briefly, when 60% to 70% of cells had evidence of SARS-CoV antigen expression, the cells were fixed in chilled acetone for 10 min at −20°C and were stored at −80°C until use.

Indirect immunofluorescence assay. SARS antibody detection was performed using indirect immunofluorescence. Sera were screened at a dilution of 1 in 10 on infected and noninfected control cells. For detection of IgG, IgA, or IgGAM antibodies, smears were incubated for 30 min at 37°C. For detection of IgM antibody, sera were preabsorbed with antihuman IgG (Gull sorb) for 10 min at room temperature before being added onto the cells followed by incubation for 3 h at 37°C. The cells were washed twice in phosphate-buffered saline for 5 min each time, and then anti-human IgG, IgA, or IgM (INOVA Diagnostic, San Diego) or IgGAM fluorescein isothiocyanate conjugates (Focus Diagnostics. Inc, Cypress, CA) were added and the cells further incubated for 30 min at 37°C. Sera positive at a screening dilution of 1 in 10 were titrated with serial twofold dilutions in parallel with the respective acute-phase serum specimen from the same patient. Sequential serum samples from each patient were assayed in the same experiment. A weak SARS CoV antibody-positive serum was included as a positive control in each run. A positive result was scored when fluorescent intensity equaled or was higher than that of the positive control. The antibody titer was taken to be the highest serum dilution giving a positive result. Epstein-Barr virus serology was performed according to a method described previously (1).

Coronavirus neutralization test. Starting with a serum dilution of 1/10, serial twofold dilutions of sera were prepared in 96-well microtiter plates. Each serum dilution (0.05 ml) was mixed with 0.05 ml of 200 50% tissue culture infectious doses of SARS CoV (HK39849) and incubated at 37°C for 1.5 h in a CO2 incubator. Then 0.1 ml of the virus-serum mixture was inoculated in duplicate wells of 96-well microtiter plates with preformed monolayers of FRhK4 cells (SARS CoV) and further incubated at 37°C. A virus back-titration was performed to assess the actual virus titer used in each experiment. A cytopathic effect was observed using an inverted microscope on days 3 and 4 postinoculation. Neutralization titer was determined as the highest dilution of serum which completely suppresses the cytopathic effect in at least half of the infected wells. The experiment was read when the virus back-titration showed the virus dose to be 100 50% tissue culture infectious doses, as expected.

Statistical analysis. Antibody titers were transformed to $\log_{10}$ and compared using the Mann-Whitney U test.

Results

Serial serum samples from patients with SARS. Serial serum samples from a cohort of 20 patients were tested by indirect immunofluorescence on cells infected with SARS CoV and human coronaviruses 229E and OC43. In addition, neutralizing antibody titers to SARS CoV were determined. The mean time (in days) to seroconversion to SARS CoV determined by IF tests when using a conjugate reacting to all human immunoglobulin subclasses (IgGAM) was 14.2 days (range, 9 to 19). Durations to seroconversion in the IgG, IgM, and IgA class-specific IF assays were 17.2 days (range of 13 to 28), 16.6 days (range of 13 to 22) and 18.3 days (range of 11 to 27), respectively. The mean time to developing neutralizing antibody was 15.4 days (range of 11 to 21).

TABLE 1. Serological-cross reactivity of sera from 20 patients with SARS[a] with human coronavirus 229E and OC43 by immunofluorescence tests

Antibody titer profile against coronavirus	No. of SARS patients (n = 20)
For both OC43 and 229E, ≥ 4-fold increase	7
For OC43 only, ≥4-fold increase	2
For 229E only, ≥4-fold increase	3
For OC43 and 229E, ≥4-fold decrease	1
For OC43 and 229E, no significant change	7

[a] All 20 patients had serological and RT-PCR confirmation of SARS CoV infection with an epidemiological link and clinical features compatible with SARS.

The mean times to seroconversion determined by the IgG, IgM, IgA, IgGAM, and neutralization test assays for patients who recovered from SARS were 17.9, 16.9, 19.1, 14.8, and 17.3, respectively, while those with fatal outcome were 17.8, 16.7, 19.2, 14.7, and 16.9.

Follow-up sera at 7 months post-onset of disease were available from 11 of these 20 patients. When compared to the highest antibody titer in the first month of illness, IgM antibody levels had fallen at least fourfold in five patients and were undetectable in three of them. Geometric mean SARS CoV IgM titers dropped from 282 at 1 month postinfection to 19 at 7 months ($P = 0.0012$). IgA antibody titers had decreased at least fourfold in five patients and remained stable in six. The geometric mean IgA antibody titers at 1 and 7 months were 97 and 35, respectively ($P = 0.11$). In contrast, only one patient showed a fourfold or greater decrease in SARS CoV IgG antibody level; the antibody level was stable in seven patients and continued to increase in three patients. Total immunoglobulin (IgGAM) titers decreased in one patient, increased in two patients. and remained stable in eight patients. Neutralizing antibody titers decreased in two patients and increased in two patients, and there was no significant change in seven patients. The geometric mean antibody titers at 1 month and 7 months post-onset of illness for IgG were 206 and 34,1 respectively ($P = 0.31$), and for IgGAM were 439 and 726, respectively ($P = 0.49$); neutralization titers remained unchanged at 124 ($P = 0.84$).

The sera from patients with SARS were also tested for antibody to OC43 and 229E by use of indirect immunofluorescence tests of virus-infected cell smears. The ranges of IgG titers against OC43 and 229E in the acute-phase serum sample were 1:10 to 1:320 and <1:10 to 1:1,280, respectively. Seven SARS patients had a fourfold or greater increase in IgG titer against both OC43 and 229E (Table 1) (Fig. 1A and B). Two patients had at least fourfold rising titers only against OC43, and three had a fourfold or greater rise in antibody only against 229E. One patient showed a decreased antibody level against both OC43 and 229E. Seven showed no significant change in antibody level against OC43 and 229E (Table 1, Fig.

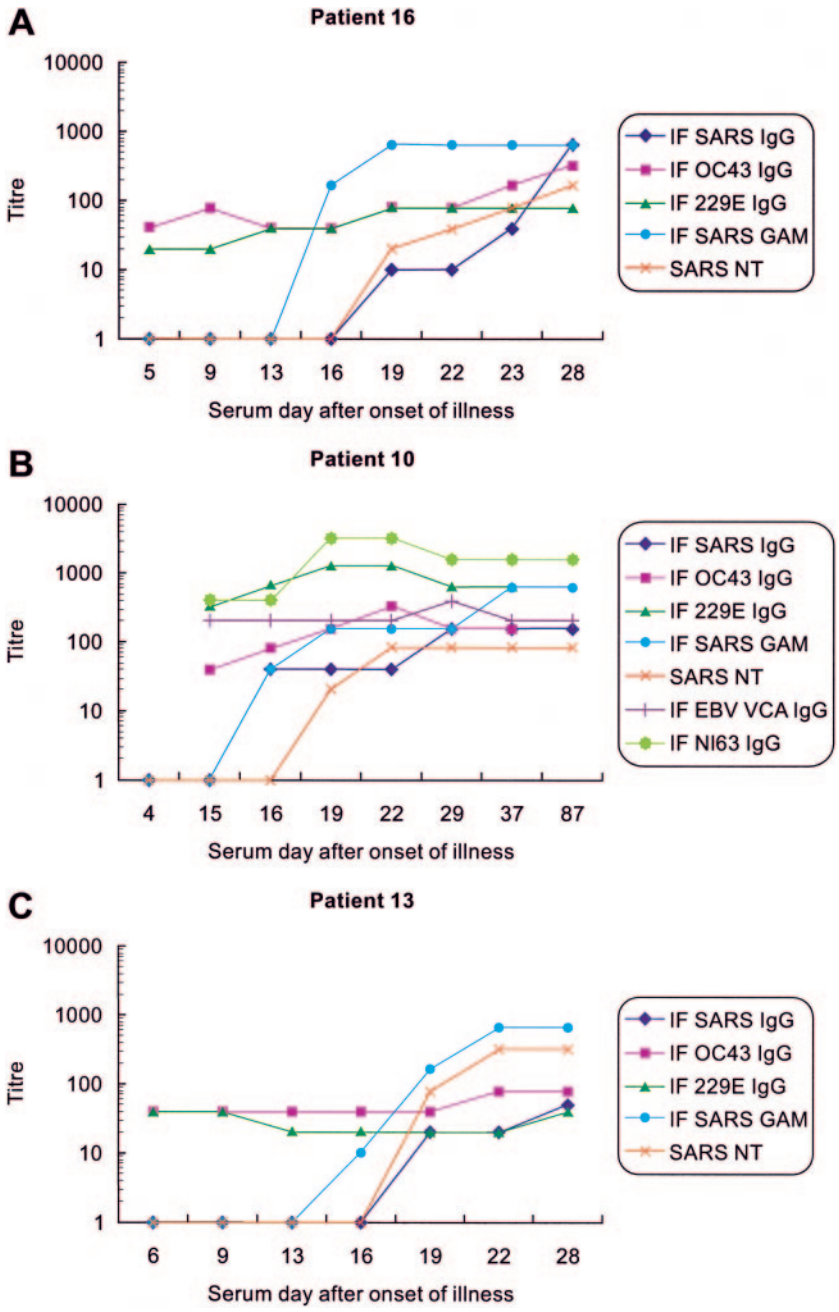

FIG. 1. (A to C) Serological profile of three illustrative patients with SARS during the first month of illness. The results of a comparison of antibody titers to SARS CoV by neutralization tests and by indirect immunofluorescence tests for IgGAM, IgG, IgM, and IgA classes are shown. For some patients, IF antibody titers to OC43, 229E, and/or NL63 are also shown.

1C). Sera from four SARS patients with a rise in IF antibody response to both OC43 and 229E and from five patients who had no cross-reactive response to these viruses were tested for IF antibody responses on NL63-infected cells. Five of these patients had a fourfold or greater increase in antibody titers to NL63; three of them also showed a rise in antibody titers to OC43 and 229E. To determine whether these cross-reactive responses were due to polyclonal activation, two patients with a significant rise of antibody to SARS CoV, 229E, OC43, and NL63 were tested for Epstein-Barr virus virus capsid antigen (VCA) IgG, which served as an antigenically unrelated control. Neither of these two patients had a significant change in Epstein-Barr virus VCA IgG titers.

Paired sera from patients with 229E and OC43 infections:. Acute- and convalescent-phase sera were available from 3 patients with 229E and 11 patients with OC43 infection. There were cross-reactive IF antibody responses between 229E and OC43 viruses (Table 2). However, all these sera remained negative by both immunofluorescent and neutralization tests for antibody to SARS CoV (data not shown).

TABLE 2. Cross-reactivity of sera from patients with primary human coronavirus 229E or OC43 infection in immunofluorescent antibody tests

Patient[a]	Primary infection	Serum status	IF IgG titer for CoV:	
			IF OC43	IF 229E
1	229E	Acute	40	<10
	229E	Convalescent	80	320
2	229E	Acute	80	20
	229E	Convalescent	320	80
3	229E	Acute	320	80
	229E	Convalescent	320	320
4	OC43	Acute	40	80
	OC43	Convalescent	160	80
5	OC43	Acute	40	160
	OC43	Convalescent	640	80
6	OC43	Acute	160	80
	OC43	Convalescent	1,280	40
7	OC43	Acute	<10	80
	OC43	Convalescent	20	40
8	OC43	Acute	40	80
	OC43	Convalescent	320	80
9	OC43	Acute	10	80
	OC43	Convalescent	80	320
10	OC43	Acute	40	80
	OC43	Convalescent	320	80
11	OC43	Acute	40	40
	OC43	Convalescent	320	20
12	OC43	Acute	<10	20
	OC43	Convalescent	1,280	320
13	OC43	Acute	<10	80
	OC43	Convalescent	160	80
14	OC43	Acute	<10	<10
	OC43	Convalescent	320	20

[a] All patients tested negative for antibodies against SARS CoV by IF and neutralization tests.

TABLE 3. Cross-reactive IF IgG responses to OC43 or 229E in patients with SARS

Coronavirus	No. of patients (total no.) showing titers rising fourfold or more against indicated CoV		
	SARS (20)	OC43 patients (11)	229E patients (3)
SARS CoV	20	0	0
OC43	9	11	1
229E	10	3	3

Discussion

Total IgGAM antibody is the antibody detectable earliest (mean, 14.2 days; range, 9 to 19 days) in patients with SARS. Of the subclass-specific assays, IgM antibodies were the earliest antibody to be detectable (mean, 16.6 days; range, 13 to 22 days). Although IgM antibody titers declined significantly during the first 7 months after infection, we demonstrated that SARS CoV IgM remains detectable in 63.6% (7/11) of patients for at least 7 months. It was previously reported that IgM antibody detected by ELISA became undetectable by 11 weeks after onset of illness (15). These differences in results may be related to differences to the sensitivity of the methods used for the serology tests.

In contrast, neutralizing antibody titers and IF IgGAM and IgG levels seem stable over the first 7 months postinfection. In addition, there was no significant difference in the kinetics of the appearance of the antibody responses between patients who survive or die.

Since serology remains the gold standard for diagnosis of SARS, it is important to explore serological cross-reactions between SARS CoV and the other human coronaviruses 229E and OC43. We show that 12 (60%) of the 20 SARS patients had fourfold rising titers to OC43, 229E, or both (Table 1). Another recent study has shown evidence of rising antibodies to OC43 and 229E in animals immunized with SARS CoV and in human patients with SARS (3). Furthermore, in the subset of patients tested, some also had rising IF antibody titers to the recently discovered NL63 coronavirus. Since most SARS patients had preexisting antibody to 229E, OC43, and NL63, SARS CoV infection appears to stimulate cross-reactive antibody responses to one or more of these viruses. This could be due to cross-reactive antigenic epitopes or to the infection resulting in polyclonal activation of antibody. However, patients who demonstrate a cross-reactive coronavirus response do not have a significant change in IF antibody to a virus of an unrelated family, e.g., Epstein-Barr virus. Thus, the presence of cross-reactive antigenic epitopes rather than polyclonal activation of antibody appears to be the explanation for these findings. Similarly, and perhaps for similar reasons, 27% of OC43-infected patients and one of three patients infected with 229E induced rising antibody titer to the other virus (Table 3). Such cross-reaction between human coronaviruses has been noted before in IF (9) and complement fixation tests (8).

On the other hand, 11 patients with recent OC43 infections and 3 with 229E infection without prior exposure to SARS CoV developed antibody to the infecting virus without inducing a cross-reacting antibody response to SARS CoV either in IF or in neutralization tests. This is in agreement with the fact that there is no IF or neutralization antibody to SARS CoV detectable in uninfected individuals (2). This lack of cross-reactive response to SARS CoV is possibly because these patients had no prior immunological memory for SARS CoV. Thus, there is less opportunity for a cross-reactive response. Given the evidence of antigenic cross-reactions between coronaviruses, it remains possible, however, that there may indeed be an increase in antibody SARS CoV titer in a patient who has previously had SARS CoV infection when the patient subsequently gets infected by OC43 or 229E. This remains to be formally demonstrated. However, awareness of this possibility is important from a diagnostic point of view. Such cross-reactions probably explain the positive results in ELISA tests based on recombinant nucleoprotein antigens (18). Furthermore, hyperimmune animal antisera to some group 1 human

and animal coronaviruses was observed to cross-react with SARS in vitro (7).

In summary, one may remain confident that seroconversion by IF or neutralization tests to SARS CoV is indeed conclusive evidence of SARS CoV infection. However, if the first available serum from a patient already has detectable antibody to SARS CoV, a rise in IF antibody titer to SARS CoV may not necessarily confirm SARS CoV infection. It remains important to obtain a better understanding of cross-reactivity of human serological responses to coronaviruses for purposes of laboratory diagnosis as well as for understanding pathogenesis and immunity.

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REFERENCES

1. **Chan, K. H., J. S. Tam, J.S. Peiris, W. H. Seto, and M. H. Ng.** 2001. Epstein-Barr virus (EBV) infection in infancy. J. Clin. Virol. **21:**57–62.
2. **Chan, K. H., L. L. Poon, V.C. Cheng, Y. Guan, I. F. Hung, J. Kong, L. Y. Yam, W. H. Seto, K. Y. Yuen, and J. S. Peiris.** 2004. Detection of SARS coronavirus in patients with suspected SARS. Emerg. Infect. Dis. **10:**294–299.
3. **Che, X. Y., L. W. Qiu, Z. Y. Liao, Y. D. Wang, K. Wen, Y. X. Pan, W. Hao, Y. B. Mei, V. C. Cheng, and K. Y. Yuen.** 2005. Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43. J. Infect. Dis. **191:**2033–2037.
4. **Drosten, C., S. Gunther, W. Preister, S. van der Werf, H. R. Brodt, S. Becker, H. Rabenau, M. Panning, L. Kolesnikova, R. A. Fouchier, A. Berger, A. M. Burguiere, J. Cinatl, M. Eickmann, N. Escriou, K. Grywna, S. Kramme, J. C. Manuguerra, S. Muller, V. Rickerts, M. Sturmer, S. Vieth, H. D. Klenk, A. D. Osterhaus, H. Schmitz, and H. W. Doerr.** 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N. Engl. J. Med. **348:**1967–1976.
5. **Gill, E. P., E. A. Dominguez, S. B. Greenberg, R. L. Atmar, B. G. Hogue, B. D. Baxter, and R. B. Couch.** 1994. Development and application of an enzyme immunoassay for coronavirus OC43 antibody in acute respiratory syndrome. J. Clin. Microbiol. **32:**2372–2376.
6. **Hsueh, P. R., C. H. Hsiao, S. H. Yeh, W. K. Wang, P. J. Chen, J. T. Wang, S. C. Chang, C. L. Kao, P. C. Yang; and SARS Research Group of National Taiwan University College of Medicine and National Taiwan University Hospital.** 2003. Microbiologic characteristics, serologic responses, and clinical manifestations in severe acute respiratory syndrome, Taiwan. Emerg. Infect. Dis. **9:**1163–1167.
7. **Ksiazek, T. G., D. Erdman, C. S. Goldsmith, S. R. Zaki, T. Peret, S. Emery, S. Tong, C. Urbani, J. A. Comer, W. Lim, P. E. Rollin, S. F. Dowell, A. E. Ling, C. D. Humphrey, W. J. Shieh, J. Guarner, C. D. Paddock, P. Rota, B. Fields, J. DeRisi, J. Y. Yang, N. Cox, J. M. Hughes, J. W. LeDuc, W. J. Bellini, L. J. Anderson, and the SARS Working Group.** 2003. A novel coronavirus associated with severe acute respiratory syndrome. N. Engl. J. Med. **348:**1953–1966.
8. **McIntosh, K., R. K. Chao, H. E. Krause, R. Wasil, H. E. Mocega, and M. A. Mufson.** 1974. Coronavirus infection in acute lower respiratory tract disease of infants. J. Infect. Dis. **130:**502–507.
9. **Monto, A. S., and L. M. Rhodes.** 1977. Detection of coronavirus infection of man by immunofluorescence. Proc. Soc. Exp. Biol. Med. **155:**143–148.
10. **Nie, Y., G. Wang, X. Shi, H. Zhang, Y. Qiu, Z. He, W. Wang, G. Lian, X. Yin, L. Du, L. Ren, J. Wang, X. He, T. Li, H. Deng, and M. Ding.** 2004. Neutralizing antibodies in patients with severe acute respiratory syndrome-associated coronavirus infection. J. Infect. Dis. **190:**1119–1126.
11. **Peiris, J. S., C. M. Chu, V. C. C. Cheng, K. S. Chan, I. F. Hung, L. L. M. Poon, K. I. Law, B. S. F. Tang, T. Y. W. Hon, C. S. Chan, K. H. Chan, J. S. C. Ng, B. J. Zheng, W. L. Ng, R. W. M. Lai, Y. Guan, K. Y. Yuen, and Members of the HKU/UCH SARS Study Group.** 2003. Clinical progression and viral load in a community outbreak of a coronavirus-associated SARS pneumonia: a prospective study. Lancet **361:**1767–1772.
12. **Peiris, J. S. M., S. T. Lai, L. L. M. Poon, Y. Guan, L.Y. C. Yam, W. Lim, J. Nicholls, W. K. S. Yee, W. W. Yan, M. T. Cheung, V. C. C. Cheng, K. H. Chan, D. N. C. Tsang, R. W. H. Yung, T. K. Ng, K. Y. Yuen, and Members of the SARS Study Group.** 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet **361:**1319–1325.
13. **Poon, L. L., Y. Guan, J. M. Nicholls, K. Y. Yuen, and J. S. Peiris.** 2004. The aetiology, origins, and diagnosis of severe acute respiratory syndrome. Lancet Infect. Dis. **4:**663–671.
14. **Schmidt, O. W.** 1984. Antigenic characterization of human coronavirus 229E and OC43 by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **20:**175–180.
15. **Shi, Y., Z. Wan, L. Li, P. Li, C. Li, Q. Ma, and C. Cao.** 2004. Antibody responses against SARS-coronavirus and its nucleocapsid in SARS patients. J. Clin. Virol. **31:**66–68.
16. **Snijder, E. J., P. J. Bredenbeek, J. C. Dobbe, V. Thiel, J. Ziebuhr, L. L. M. Poon, Y. Guan, M. Rozanov, W. J. Spaan, and A. E. Gorbalenya.** 2003. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J. Mol. Biol. **331:**991–1004.
17. **Van den Hoek, L., K. Pyrk, M. F. Jebbink, W. Vermeulen-Oost, R. J. Berkhout, K. C. Wolthers, P. M. Wertheim-Van Dillen, J. Kaandorp, J. Spaargaren, and B. Berkhout.** 2004. Identification of a new human coronavirus. Nat. Med. **10:**368–373.
18. **Woo, P. C., S. K. Lau, B. H. Wong, K. H. Chan, W. T. Hui, G. S. Kwan, J. S. Peiris, R. B. Couch, and K. Y. Yuen.** 2004. False-positive results in a recombinant severe acute respiratory syndrome-associated coronavirus (SARS-CoV) nucleocapsid enzyme-linked immunosorbent assay due to HCoV-OC43 and HCoV-229E rectified by Western blotting with recombinant SARS-CoV spike polypeptide. J. Clin. Microbiol. **42:**5885–5888.
19. **Woo, P. C. Y., S. K. P. Lau, C. M. Chu, K. H. Chan, H. W. Tsoi, Y. Huang, B. H. L. Wong, R. W. S. Poon, J. J. Cai, W. K. Luk, L. L. M. Poon, S. S. Y. Wong, Y. Guan, J. S. M. Peiris, and K. Y. Yuen.** 2005. Characterization and complete genome sequence of a novel coronavirus HKU-1 from patients with pneumonia. J. Virol. **79:**884–895.