Brief report

A retrospective serological study of severe acute respiratory syndrome cases in Guangdong province, China

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Severe acute respiratory syndrome (SARS) is a life threatening, upper respiratory disease. Its cause is a coronavirus, SARS-CoV. Since its emergence in 2003 in China, SARS has affected more than 8000 patients and caused 776 deaths in 26 countries.[1] A reemergence of SARS occurred in Guangdong province, in which the first case was confirmed on January 5, 2004 and three more reported the following month.

To better understand the immunological characteristics of the SARS pandemic, we studied SARS-CoV neutralizing antibody titres of four reemerging SARS patients using SARS-CoV strains (Z2-Y3 and F69) isolated from two previous cases. Their neutralizing antibody profiles were compared with those of fourteen SARS cases infected in Guangdong province prior to 2004, including the first identified case on November 16, 2002.

METHODS

Epidemiological investigation

Medical records and close contacts of the SARS patients, provided by Guangdong CDC, were analysed. Laboratory safety procedures were carefully reviewed.

Serum collection

Sera were collected from the four reemerging SARS cases (A, B, C and D) and from fourteen SARS cases before 2004 at different time points (Tables 1 and 2).

SARS-CoV strains

SARS-CoV strain Z2-Y3 (NCBI/Genbank: AY394989) was previously isolated from the pharynx swab of a 35 years old female medical faculty member (Guangdong province) hospitalized on February 5, 2003, and diagnosed with the infection on February 12 of the same year. SARS-CoV strain F69 (NCBI/Genbank: AY313906) was previously isolated from sputum specimen of another case in Guangdong. The patient was hospitalized on April 3, 2003 and confirmed with SARS on April 9, 2003. Both strains were isolated from Vero-E6 cells and identified as SARS-CoV virus by electron microscopy, reverse transcription polymerase chain reaction and sequence analysis. SARS-CoV virus Z2-Y3 and F69 strains were sequenced and compared, showing certain differences (Table 3).

Determination of neutralizing antibody titre

TCID50 SARS-CoV was titrated by Reed-Muench method.[2] Titration results showed that Z2-Y3 and F69 strain titres reached 6.5log TCID50/25 μl and 6.6log TCID50/25 μl, respectively. Neutralizing antibody assay was carried out according to standard procedure (WHO, 1997. Manual for the virological investigation of polio. WHO/EPI/GEN/97.01). Sera from the four reemerging cases were inactivated at 56˚C for 30 minutes, and incubated with 100 TCID50 of both Z2-Y3 and F69 SARS-CoV strains at 36˚C for 2 hours. The same method was applied for sera from the fourteen earlier cases using Z2-Y3 strain isolated during the original epidemic. Vero-E6 cells (10^4 cells/ml) were added to the neutralizing mixture. Plates were incubated at 36˚C for 5−7 days and examined with an inverted microscope for the appearance of cytopathic effects.

RESULTS

To characterize the neutralizing antibody profiles of the four reemerging SARS cases, sera were collected at various times and incubated with two strains of SARS-CoV (Z2-Y3 and F69) isolated from patients infected in early 2003. The neutralizing antibody titres of 4 reemerging cases peaked within 11−13 days at a lower level (1: 160−1: 640), and then rapidly dropped after a short period of plateau (Fig). This observation is in sharp contrast to the neutralizing antibody titres of SARS

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Table 1. Human sera of 4 reemerging SARS cases in Guangdong province in 2004

Case	Sex	Age (years)	Date of onset	Date of diagnosis	Sample collection (Days after onset)	Epidemiology (contact)	
						Wild animal	Hospital
A	M	32	Dec. 28, 2003	Jan. 5, 2004	6,7,8,9,10,11,12,13,15,17	−	−
B	F	20	Dec. 28, 2003	Jan. 17, 2004	7,8,11,19,22	+	−
C	M	35	Dec. 31, 2003	Jan. 17, 2004	8,10,11,13,18	−	−
D	M	40	Jan. 7, 2004	Jan. 26, 2004	9,12,16	−	+

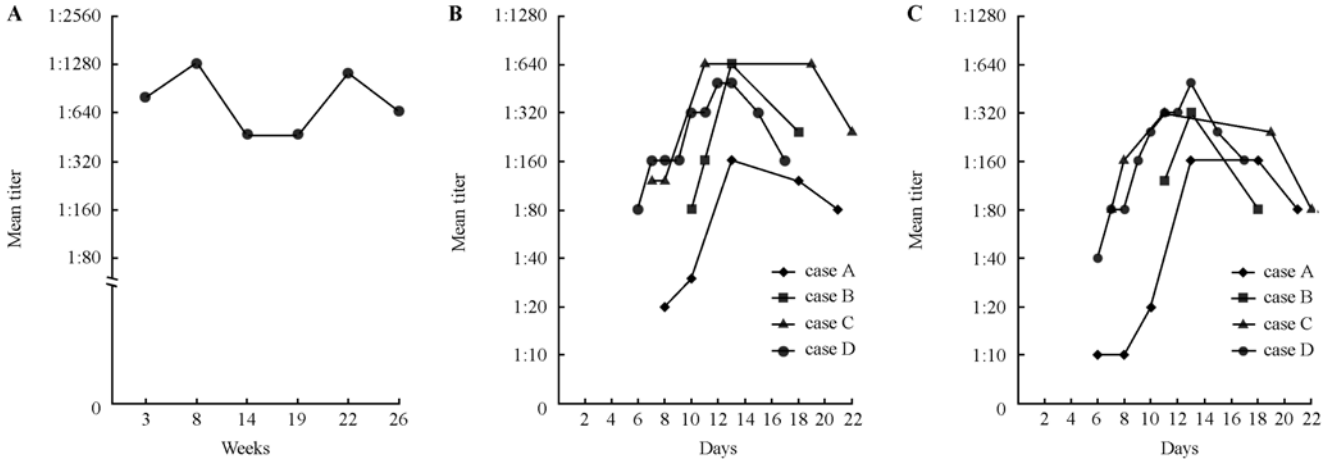

Fig. The neutralizing antibody titres of SARS cases. **A:** Neutralizing antibody titre of 14 SARS cases in retrospective screening; **B:** Neutralizing antibody titter of 4 reemerging SARS cases by SARS-CoV strain Z2-Y3; **C:** Neutralizing antibody titter of 4 reemerging SARS cases by SARS-CoV strain F69.

Table 2. Human sera of SARS patients in early 2003

Case	sex	Age(years)	Date of onset	Days from onset to sample collection
1	M	52	Jan. 7, 2003	17
2	M	23	Feb. 5, 2003	21
3	F	32	Jan. 2, 2003	50
4	M	44	Jan. 26, 2003	54
5	F	36	Feb. 7, 2003	94
6	F	36	Feb. 5, 2003	96
7	F	21	Feb. 2, 2003	99
8	M	31	Apr. 4, 2003	130
9	M	39	Jan. 5, 2003	137
10	M	32	Jan. 5, 2003	138
11	M	40	Dec. 16, 2002	151
12	M	35	Dec. 15, 2002	153
13	M	50	Nov. 27, 2002	174
14	M	45	Nov. 16, 2002	181

M: male; F: female.

cases prior to 2004 that maintained a high level (1:320−1280) for a long period (ranging from 17-181 days).

DISCUSSION

SARS triggered a worldwide panic in 2003, but has been under control since then. A coronavirus (SARS-CoV) has been isolated and identified as the cause. The unavoidable phenomenon of genetic and phenotypic variants in RNA viruses,[3] observed in SARS-CoV,[4] requires persistent surveillance of the virus for fear of new lethal mutation, which was emphasized by sporadic reemerging cases in Singapore and China.

By a retrospective study, we characterized the neutralizing antibody profile of fourteen SARS cases prior to 2004 and another four that reemerged in Guangdong province, China, to track recent serological changes of SARS. Analysis of the neutralizing antibody profiles demonstrated the immune response of 14 primary cases was stronger and more persistent compared with the reemerging cases: SARS-CoV may be capable of activating strong immunity within humans during the early phase. However, the virus may adapt to humans and have activated relatively weak immunity within the four reemerged cases. Interestingly, the findings of our neutralization assay can be indirectly confirmed through other studies of antibody responses to SARS-CoV using enzyme linked immunosorbent assay (ELISA).[5]

Table 3. Complete genomic sequence comparison between F69 and Z2-Y3

Locus	1–15	2015	3852	5455	6247	6760	7347	7777	8094
F69	N1	C	C	T	C	G	A	G	T
Z2–Y3	—	T	T	C	T	A	C	A	C
Locus	8591	9333	10265	11493	13470	14186	16959	17565	20374 20383
F69	G	C	T	T	A	T	T	T	N2
Z2-Y3	A	A	C	C	G	A	C	G	—
Locus	21732	22233	24706	25275	25309	26488	27403	29358	
F69	G	T	G	G	G	G	T	G	
Z2-Y3	A	C	A	A	A	T	C	A	

N1: atattaggtttttac; N2: caagaatgta; —: no nucleotide.

The clinical symptoms and epidemiological characteristics of the 4 reemerging SARS cases also distinguish them from the previous cases.[6] During the early stages of the SARS epidemic, SARS rapidly spread through close contact with an infected person and carried high mortality rates.[7] However, among the 4 reemerging cases, all patients survived and none showed evidence of transmission to others. Though these phenomena might be attributed to the fact that we already had clinical experience from the previous SARS epidemic, other factors involved should also be noted, for example the change of virulence due to the adaptable variation of virus within humans.[8]

Even though for these 4 reemerging cases, the symptoms were weaker and prognoses were better than the previous cases, one cannot be optimistic, since the latest emergence of SARS occurred in April 2004, in Beijing and Anhui province and spread. From an epidemiologic perspective, these latest cases of April 2004 bear more similarity with the SARS cases prior to 2004 than do the four cases discussed in this study.

Of these 4 reemerging cases, 2 patients had no history of recent hospital admission or contact with wild animals. It is logical to suppose reservoirs, yet unidentified, are involved in viral transmission and require further investigation.

Study of the strain sequences yields differences in the genome between Z2-Y3 strain and F69 strain as shown above (Table 3). The sequence analysis indicated that the gene variation do not change the important neutralizing epitope, which is confirmed by the current result that no significant difference in the neutralizing antibody level between the two strains. By comparing the presentation and prognosis of fourteen earlier cases and four reemerging cases, we may suppose that the mutation of SARS-CoV might be restricted to those genes responsible for variation of the virulence. A well defined characterization of the molecular epidemiology, antigen mutation, and epitopes from the 4 reemerging SARS cases in Guangdong would be valuable to the development of SARS vaccine.

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