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Short communication

Antibody responses against SARS-coronavirus and its nucleocaspid in SARS patients

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Abstract

Background: SARS-Cov is the etiologic agent of severe acute respiratory syndrome. An understanding of the antibody responses to the viral components is very important for diagnosis and vaccine development. *Objective:* The spectrum of SARS-specific antibody profiles in SARS patients was investigated from 7 to 210 days after the onset of the symptoms. *Study design:* Serial serum samples from 14 SARS patients were isolated from 7 to 210 days after the onset of the symptoms, and were tested for anti-viral IgG and IgM by indirect immunofluorescence tests (IFA), anti-nucleocaspid antibody by ELISA tests and viral neutralization. *Results:* Anti-viral (IgG) and anti-nucleocaspid antibodies were observed in 13 of 14 patients at 14 days after the onset of symptoms, and in all 14 patients at 30–210 days thereafter. Anti-viral antibody (IgM) was detected maximally at 30 days, later than that for the IgG class. IgM antibody declined and became undetectable between 60 to 180 days after the onset of the symptoms. Neutralizing viral antibodies were demonstrated in the sera from all of the patients with SARS symptoms. *Conclusions:* Anti-viral IgG, IgM, and anti-nucleocaspid antibodies were detected 7–30 days in patients after the onset of SARS symptoms. Anti-viral IgM antibodies disappeared earlier than IgG. Viral neutralization was demonstrated in the sera from the convalescent patients.

Keywords: Severe acute respiratory syndrome; Antibody; Coronavirus

1. Introduction

Severe acute respiratory syndrome (SARS) was first reported in Guangdong Province, China in November 2002 followed by spread to more than 30 countries worldwide (Lee et al., 2003). A newly isolated coronavirus (SARS-CoV) was found to be the etiological agent of SARS (Drosten et al., 2003), and the genome of the virus was soon sequenced (Marra et al., 2003). The development of vaccines against SARS-CoV have subsequently been pursued in several laboratories. It is of great importance to identify the characteristics of host antibodies against SARS virus and its components, to aid in vaccine development and diagnosis of the viral infection (Chen et al., 2004; Li et al., 2003).

2. Methods

Fourteen patients aged from 22 to 73 years old (median of 45 years), were diagnosed as probable SARS patients based on WHO criteria in February 2003. Serial serum samples were collected at 7–210 days after the onset of symptoms, and were subjected to antibody analysis. Anti-viral antibodies (IgG, IgM) were assayed by indirect fluorescence (IF) kits developed by Beijing Institute of Microbiology and Epidemiology and approved by State Drugs Administration of China (Si et al., 2003). 20 μl diluted sera (1:20 or greater dilution) were added onto slides containing monolayers of SARS-CoV-infected Vero cells and non-infected Vero cells as a negative control. The slides were incubated at 37 °C for 30 min, washed three times with PBS (phosphate buffered saline), and then FITC-labeled anti-human IgG or FITC-labeled anti-human IgM were added for 30 min. The slides were washed as before, and fluorescence was detected with a Zeiss Axiovert 200 M microscope. The dilution for cut off is 1:20. Anti-nucleocaspid (N protein, NP) total Ig

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was assayed by an antigen-capturing ELISA (AC-ELISA) co-developed by Sina-American Biotechnology Co. Ltd. and Beijing Institute of Biotechnology (Shi et al., 2003). For this assay, 100 μl of serum samples were added to wells coated with recombinant N protein, the plate was incubated at 37°C for 30 min, and then washed five times with PBS containing 0.05% Tween 20. 100 μl HRP (Horse radish peroxidase)-conjugated N protein was added and the plate was incubated for another 30 min followed by washing as above. Then 100 μl of TMB substrate solution (0.1 mg/ml tetramethylbenzidine hydrochloride, 0.01% H_2O_2 in 0.1 M acetate buffer pH 5.8) was added and incubated at 37 °C for 20 min, The reaction was terminated by adding 50 μl 2 N sulfuric acid and the absorbance at 450 nm was determined. The maximum dilution of the serum at which a positive signal was obtained (IFA), or an absorbance at 450 nm wavelength greater than 0.15 (cutoff value) was given, was defined as antibody titer. The data were analyzed by SPSS11.0 software and the geometric means of seropositive samples were employed in all statistics.

Neutralization antibody was assayed by the following protocol: 1 ml diluted serum was mixed with 1 ml DMEM medium containing 2×10^6 SARS-CoV (strain BJ-1) virus, and incubated at 37 °C for 1 h in a water-bath. Then the mixture was aliquotted to 8 wells (200 μl each) of Vero E6 cell monolayers grown in 96-well microtiter plates. The plate was incubated at 37 °C, 5% CO_2 for 7 days. The highest dilution of the serum at which 50% of the wells were protected from viral cytopathic effect was considered to be the neutralizing titer.

3. Results and discussion

Seroconversion was observed in 13 of the 14 patients either by anti-viral IgG (FIA) or by antigen capturing anti-N protein assay by 14 days after the onset of the symptoms. In contrast, anti-viral IgM was detectable only in 10 patients by14 days, and in 13 patients by 30 days after the onset of the symptoms. By comparison, anti-viral IgG and anti-N protein antibodies were detected in all the patients from 30 to 210 days after the onset of the symptoms. Antiviral IgM was shown to be negative in 4, 8, 12 and all 14 patients by day 60,120,180 and 210 days post disease onset, respectively. These results may indicate that antiviral IgG may correlate better with viral neutralization. Also, the recombinant nucleocaspid protein based antigen-capturing ELISA was at least as sensitive as the anti-viral IgG(IFA) assay.

Anti-viral IgG and anti-Nucleocaspid antibodies were detectable earlier than anti-viral IgM antibodies, and the highest antibody titer was reached at 120 days (anti-viral IgG) and 180 days (anti-N protein) after the onset of the symptoms. The titer decreased slowly after that time point, and high titers of antibodies were observed in all patients 210 days after the onset of symptoms. Geometric mean values of the antibodies were shown in Fig. 1. In contrast, a significantly lower titer of anti-viral IgM antibody was found with a peak titer at 30 days. Moreover, anti-viral IgM could not be detected in any of the 14 patients 210 days after the onset of the symptoms.

To understand potential viral neutralization in convalescent patients against the infection by the virus, neutralizing antibodies were assayed in six convalescent patient's sera collected 20, 30, 60, 120, and 210 days after the onset of symptoms. It was found that, in all six patients, neutralizing antibodies were detected. The geometric means of the neutralization titers on day 20, 30, 60, 120 and 210 was 1:150, 1:475, 1:400, 1:200 and 1:200, respectively. These data indicate that viral neutralization is a feature of SARS infection, and the duration of the viral neutralization is greater than 7 months.

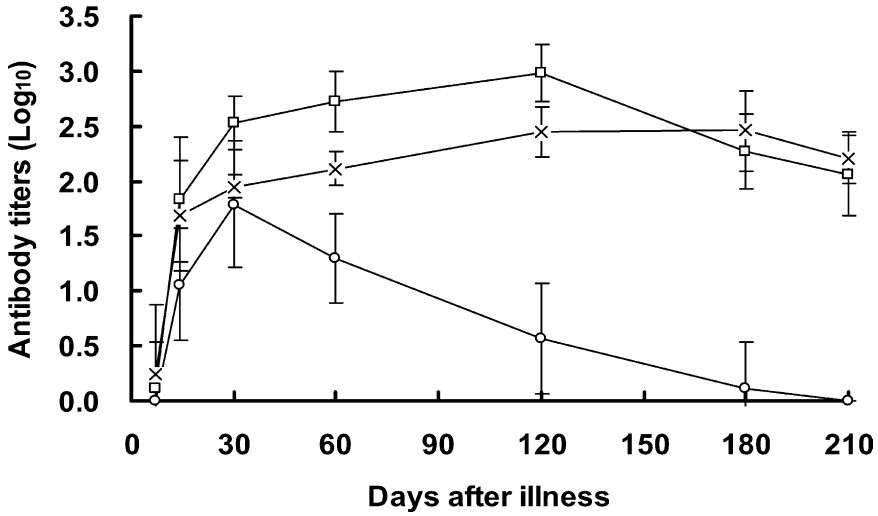

Fig. 1. Geometric means of immunofluorescent antibody titers for IgG (□) and IgM (○) and anti-nucleocaspid antibody titres (×) in 14 probable SARS patients. The error bars correspond to 1 S.D. Sera were collected from 7 to 210 days after the onset of the symptoms. Anti-viral IgG and IgM are detected by IFA and anti-NP total Ig by antigen-capture ELISA.

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