Correspondence

Natural Course of Severe Acute Respiratory Syndrome–Associated Coronavirus Immunoglobulin after Infection

To the Editor—In Chen et al.'s recent study, severe acute respiratory syndrome (SARS)–associated coronavirus (SARS CoV) IgG was shown to be persistent for up to 60 days [1]. Their results suggested that production of this antibody is dependent on $CD4^+$ cells and might play a role in protective immunity against SARS CoV. On the other hand, IgM has been found to decrease and become undetectable 11 weeks into the recovery phase [2]. Whether survivors of SARS can have persistent antibody and lifelong immunity against SARS CoV is unknown.

We conducted a 1-year prospective study to investigate the natural course of SARS CoV immunoglobulin titer in 62 survivors of SARS and in 1 asymptomatic infected health-care worker, as described in our previous reports [3, 4]. All of the serologic tests were performed at the Government Virus Unit, Hong Kong, China, following a standard protocol. Serum samples were diluted 1:25, and 15 μL of the diluted samples were incubated by use of microscopic slides coated with SARS CoV–infected fetal rhesus kidney cells in a moist chamber for 30 min at 37°C. The slides were then washed with 2 changes of Tween 20 (Sigma). Fifteen microliters of polyvalent anti–human immunoglobulin labeled with fluorochrome was added, and the slides were incubated again for 30 min at 37°C. This was followed by another wash with 2 changes of Tween 20. The slides were then examined by use of a fluorescence microscope, under a low-power field (20×). Any positive signals showing cytoplasmic fluorescence were confirmed by examination under a high-power field (40×), and all tests with indeterminate results were repeated with uninfected cells, to exclude nonspecific reactions. Results were quantified by use of serial titrations of serum samples from patients and were reported as titers (<25, 25, 50, 100, 200, 400, 800, 1600, and 3200).

For all of the survivors of SARS in our study, serum samples were collected on the day of admission to the hospital and 15 days, 1 month, 3 months, 6 months, 9 months, and 12 months after the onset of SARS symptoms. The mean age of the survivors of SARS was 37.07 years (SD, 12.96 years), and the male:female ratio was 0.82. On admission to the hospital, all of them had a baseline SARS CoV immunoglobulin titer <25. Fifteen days after the onset of symptoms, the mean SARS CoV immunoglobulin titer was 252.8 (figure 1*A*); at 1 and 3 months after the onset of symptoms, the mean SARS CoV immunoglobulin titer had increased to 613.3 and 880.3, respectively. Afterward, a gradual decrease in the mean SARS CoV immunoglobulin titer was observed, to 167.7 at 12 months after the onset of symptoms (i.e., a 5.3-fold decrease in mean titer at 12 months after the onset of symptoms, compared with the mean titer at 3 months after the onset of symptoms). For the asymptomatic infected health-care worker, serum samples were collected 1, 3, 6, 9, and 12 months after the first day she was deployed to the SARS ward. Her first SARS CoV immunoglobulin titer was 400 (figure 1*B*), which decreased to 50 at 3 and 6 months after deployment (i.e., an 8-fold decrease in titer). At 9 and 12 months after deployment, her SARS CoV immunoglobulin titer was only 25.

A previous study has reported that, over time, neutralizing antibody against other CoVs can decay to an undetectable level

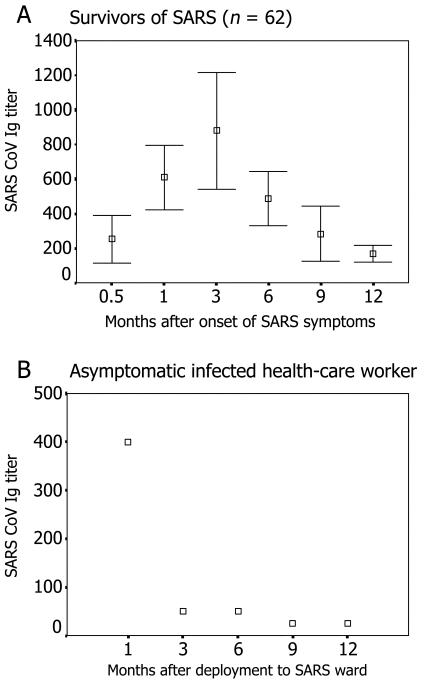

Figure 1. Severe acute respiratory syndrome–associated coronavirus immunoglobulin (SARS CoV Ig) titers, measured over the course of 1 year in 62 survivors of SARS (shown are mean titers with 95% confidence intervals) *(A)* and in 1 asymptomatic infected health-care worker *(B)*.

[5]. In SARS, the human antibody against the S1 spike protein of SARS CoV has been shown to have a neutralizing effect [6]. Our findings have shown that survivors of SARS experience a minimum 5-fold decrease in SARS CoV immunoglobulin titer over 9 months, and the asymptomatic infected health-care worker in our study experienced an even more rapid decrease in SARS CoV immunoglobulin titer. It remains to be seen whether SARS CoV immunoglobulin will finally disappear in infected persons. The progressive decrease in SARS CoV immunoglobulin titer implies that, over time, infected persons may experience a decrease in protective immunity against SARS CoV. Convalescent serum has been used to treat patients with

SARS in Hong Kong and other parts of China, because it is expected that survivors of SARS have high levels of SARS CoV immunoglobulin [7]. On the basis of the findings of our study, convalescent serum ideally should be collected from donors ~3 months after the onset of symptoms, the time at which the yield of SARS CoV immunoglobulin would be the highest.

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Reply to Tso et al.

To the Editor—We appreciate the letters from Tso et al. [1, 2] that expand on our study [3] of the serologic profile of severe acute respiratory syndrome (SARS). On the basis of the findings of our study, we are certain that IgG antibody persists for at least 60 days after the onset of symptoms. This is consistent with Tso et al.'s results, which, despite the different assays used, indicate that SARS-associated coronavirus (SARS CoV) immunoglobulin titers continue to increase from 15 days to 3 months after the onset of symptoms.

Interestingly, the rate of seroconversion differs in the 2 studies, with an 83% rate of seroconversion reported in our study and a 100% rate of seroconversion reported in Tso et al.'s study [2]. This may have been a consequence of the different populations of patients and of the different assays used in the studies. Tso et al. selected 62 survivors from a group of 267 patients with SARS, among whom 78% had a 4-fold increase in SARS CoV immunoglobulin or a single titer of $\geq$100 during their first 3 months of follow-up [2, 4]. This 78% rate of seroconversion in the 267 patients [4] was close to our observations of 75% on day 21 and 83% on day 60. According to several reports, the immunofluorescent assay used by Tso et al. is more sensitive than the ELISA used in our study [5–7]. Nevertheless, ELISA provides similar results and remains the most convenient and reliable test in both clinical practice and epidemiologic studies.

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