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Colonization of Severe Acute Respiratory Syndrome-Associated Coronavirus Among Health-Care Workers Screened by Nasopharyngeal Swab*

Hsin-Tsung Ho, MD, PhD; Mau-Sun Chang, PhD; Tsai-Yin Wei, MS; Wen-Shyang Hsieh, MS; Chia-Chien Hung, MS; Huei-Mei Yang, BS; and Yen-Ta Lu, MD, PhD

Study objectives: To report the efficacy and findings of a large-scale preventive screening program for severe acute respiratory syndrome-associated coronavirus (SARS-CoV) using amplification of the virus from a nasopharyngeal swab (NPS) obtained from the health-care workers (HCWs).

Design: A prospective observational study.

Setting: A medical center in Taiwan.

Participants: Two hundred thirty HCWs.

Intervention: NPS examination for the presence of SARS-CoV by two nested reverse transcription-polymerase chain reaction (RT-PCR) assays.

Measurements and results: During the outbreak of severe acute respiratory syndrome (SARS), NPS polymerase chain reaction screening of HCWs for SARS-CoV was performed. SARS-CoV was examined by two nested RT-PCRs and a quantitative RT-PCR. Serum-specific antibodies were assessed by enzyme immunoassay and indirect immunofluorescence. We monitored 230 HCWs, including 217 first-line HCWs and 13 non–first-line HCWs. One hundred ninety first-line HCWs and 13 non–first-line HCWs had negative results in both nested RT-PCR assays. Two first-line HCWs who were positive on both nested RT-PCR assays had SARS. They had 16,900 ± 7,920 copies (mean ± SD) of RNA per milliliter in the NPS and had detectable anti-SARS antibodies. The remaining 25 first-line HCWs were negative for the first nested RT-PCR but positive for the second nested RT-PCR. Their corresponding titers were 338 ± 227 copies of RNA per milliliter; antibodies developed in none of these 25 HCWs. The expression and function of angiotensin-converting enzyme-2 were not different among these HCWs. This study shows that colonization of SARS-CoV occurred in 25 of 217 well-protected first-line HCWs on a SARS-associated service, but they remained seronegative.

Conclusion: With the second RT-PCR assay more sensitive than the first RT-PCR assay, we are able to show that approximately 11.5% of well-protected HCWs exposed to SARS patients or specimens may have colonization without seroconversion. Only those with significant clinical symptoms or disease would have active immunity. Thus, regular NPS screening for nested RT-PCR assays in conjunction with a daily recording of body temperature in all first-line HCWs may provide an effective way of early detection.

(CHEST 2006; 129:95–101)

Key words: immunology infection; nosocomial infection; viral disease

Abbreviations: ACE = angiotensin-converting enzyme; bp = base-pair; EIA = enzyme immunoassay; HCW = health-care worker; IIFT = indirect immunofluorescence test; NPS = nasopharyngeal swab; PBMC = peripheral blood mononuclear cell; RT-PCR = reverse transcription-polymerase chain reaction; SARS = severe acute respiratory syndrome; SARS-CoV = severe acute respiratory syndrome-associated coronavirus

It has been reported that as many as 21% of a total of 8,098 patients worldwide confirmed to have probable severe acute respiratory syndrome (SARS) from November 2002 to July 2003 were health-care workers (HCWs).1 The nosocomial spread of the virus in large hospitals was the major epidemic feature of early SARS outbreaks, causing high morbidity and mortality among HCWs.2 A convincing
transmission route of this emerging disease remains to be determined, but it is believed that the illness was mainly transmitted by close contact with contaminated droplets. Despite the World Health Organization recommendation that all HCWs should use personal protective equipment, it was later shown that clusters of cases still occurred among protected HCWs. Subclinical infection among some HCWs who might harbor the virus but in undetectable levels have been suggested, although as yet unproven as a possible means of transmission. Understanding how SARS can be spread is imperative, since enforcing the early isolation and stringent protection of potential SARS cases would greatly aid the prevention of in-hospital transmission. To prevent nosocomial spread of SARS, additional preventive measurements were implemented for the first-line HCWs in Mackay Memorial Hospital, including centralization of accommodation for off-duty HCWs and the early detection of SARS-associated coronavirus (SARS-CoV) by performing nasopharyngeal swab (NPS) and reverse transcription-polymerase chain reaction (RT-PCR) testing. The aim of this prospective study was to report the efficacy of NPS screening for detection of subclinical infections.

**Materials and Methods**

**NPS Screening of HCWs**

The study was approved by the hospital institutional review board, and informed consent was obtained from all participants. The Mackay Memorial Hospital in Taiwan is a 2,000-bed teaching hospital that employs 4,500 physicians, nurses, allied health professionals, and clerical staff members. Between April 27 and June 16, 2003, there were 96 suspected SARS patients and 71 probable SARS patients treated in our hospital. During this period, we monitored 230 HCWs, including 217 first-line HCWs and 13 non–first-line HCWs. The first-line HCWs were those with close contact with SARS patients, including medical staff in the emergency department, SARS ward, respiratory care units, and personnel who manage laboratory specimens from SARS patients. Other employees were classified as non–first-line HCWs if they had no contact history with SARS patients or specimens; this included housekeeping staff and transporting personnel. The first-line HCWs attending to patients with suspected or probable SARS were required to wear gloves, gowns, goggles, and N-95 masks. All participants were required to complete questionnaires describing their workplace, contact history with SARS patients or specimens, and symptoms experienced during the study period. Mild symptoms are defined as clinical symptoms that do not require medical treatment or can be treated with the-over-the-counter medications. Since the titer of SARS-CoV was noticed to increase in nasopharyngeal aspirates of patients between day 5 and day 10 as reported by Peiris et al, we decided to perform NPS screening of the first-line HCWs at the end of 1 week of SARS-associated service. The subjects with initially positive NPS results were required to stay isolated in the centralized dormitory until the repeat sampling results became negative and/or the subject had no fever for 3 days. The first serum sample was drawn at the 30- to 48-day interval, and the second sample was collected at the 64- to 75-day interval after the NPS was obtained. Baseline samples had been drawn from HCWs for the serologic test before their SARS-associated service. Anonymous processing of samples was performed to preserve the confidentiality of the HCWs. The critical laboratory information was only reported to the superintendents of medical technicians, nurses, and physicians.

**Nested RT-PCR Assays Specific for SARS-CoV**

Each NPS specimen was collected in 2 mL of reagent (Trizol; Invitrogen; Carlsbad, CA), and total RNAs were extracted according to the instructions of the manufacturer (GIBCO/BRL; Grand Island, NY). The extracted viral RNA of each specimen was resuspended in 100 μL of ribonuclease-free water. The RNA was reversely transcribed with random hexamers (Abgene; UK), and reactions contained 12.5 μL of 2× buffer concentrate, 3.6 mmol/L of magnesium sulfate, 0.5 μL of each primer, 0.5 μL of reverse transcriptase/Taq DNA polymerase mixture, and 5 μL of RNA in a total volume of 25 μL. The complementary DNA of every specimen was subjected to both the first and second nested RT-PCR assays. The first nested RT-PCR assay used the primer pairs IN-6/IN-7 and Cor-p-F2/Cor-p-R1. Within the open reading frame 1b of the coronavirus polymerase gene, the 440 base-pair (bp) segment was amplified with a broadly reactive genus-specific primer pair, IN-6 (+) 5’GGTGGGAGCTATCCTAAAGTGTGA3’ and IN-7 (-) 5’CCATCATCGATAAGTACATCAT3’. The complementary DNA was further amplified with a SARS-specific primer pair Cor-p-F2 (+) 5’CTAACAGCTTAAAGATAATGGG3’ and Cor-p-R1 (-) 5’CAGGTTAACGGTAAACTCTCGC3’. The size of the first nested RT-PCR product was 306 bp (lane 2, Fig 1). Similarly, a duplicate aliquot of complementary DNA for each specimen was also amplified with the second nested RT-PCR using the sets of primers Cor-p-F2/Cor-p-R1 and then Cor-p-F3/Cor-p-R2. The Cor-p-F3 (+) 5’GCCTTCCTTCTTGCTTCTGCGC3’ and Cor-p-R2 (-) 5’CCATTTCTTATAGAGACTC3’ were another pair of SARS-specific primers. The size of the second nested RT-PCR product was 306 bp (lane 4, Fig 1). Positive and negative RT-PCR controls were included in each run. The specificity of nested RT-PCR was confirmed by sequencing the positive amplified products obtained through agarose gel electrophoretic separation and compared with the reference sequence of SARS-CoV.8 Thermocycling of reaction mixtures was completed in a thermocycler (model 2400; Perkin Elmer-Applied Biosystems) pro-

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*From the Department of Laboratory Medicine, the Department of Medical Research, and the Division of Chest Medicine, Department of Internal Medicine, Mackay Memorial Hospital; and Department of Respiratory Care, Taipei Medical University; and Mackay Medicine, Nursing and Management College, Taipei, Taiwan.

This study was supported by the Taiwan National Science Council (Grant NSC92–2751-B-195–001-Y) and the National SARS Research Program (SCLI01).

Manuscript received March 24, 2005; revision accepted July 11, 2005.

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Correspondence to: Yen-Ta Lu, MD, PhD, Division of Chest Medicine, Department of Internal Medicine, Mackay Memorial Hospital, 92, Sec 2, Chung-Shan North Rd, Taipei, 10449, Taiwan; e-mail: ytlhl@ms2.mmh.org.tw

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Original Research
Quantitative RT-PCR was established with SARS-specific gene primers and 5’-nuclease probe (Assays-on-Demand Gene Expression Products; Applied Biosystems; Foster City, CA). The 381-bp target fragment of SARS-CoV RNA was transcribed and amplified (ABI PRISM 7700 sequence detection system; Applied Biosystems). Plasmids (a gift from the Center for Disease Control and Prevention Products; Applied Biosystems; Foster City, CA) were used with the target sequence to generate the standard curve. Quantity of messenger RNA was determined by real-time fluorescence measurements performed in a total volume of 25 μL (TaqMan one-step RT-PCR Master Mix Reagent; Applied Biosystems). The reaction mixtures were heated to 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Real-time fluorescence measurements were obtained, and a cycle threshold value for each sample was calculated by determining the point at which the fluorescence increased above a threshold limit. A standard curve of the cycle threshold values obtained from the serial dilutions of the standard was compiled. The coefficient of linear regression and the slope for each standard curve were calculated. The cycle threshold values from samples were plotted on the standard curve for calculating the number of genomes.

Serologic Tests for SARS-CoV-Specific IgG and IgM

Serum samples were stored at −70°C and subsequently tested for IgG and IgM for SARS-CoV by using enzyme immunoassay (EIA). The wells of microtiter plate (General Biologicals Corporation; Hsinchu, Taiwan) were coated with the purified recombinant glutathione S-transferase-nucleocapsid protein. Using the antigen-antibody-antigen sandwich technique, a recombinant glutathione S-transferase-nucleocapsid protein-horseradish peroxidase conjugate was prepared for the detection of antibodies. Serum samples from hospitalized patients with SARS and from unexposed laboratory personnel were included as positive and negative control samples, respectively. All the serum samples were further confirmed by use of an indirect immunofluorescence test (IIFT) for IgG or IgM specific for SARS-CoV. The test utilized BIOCHIP slides (Euroimmun; Berlin, Germany) containing SARS-CoV–infected cells and noninfected cells positioned side by side in each reaction field. The presence of serum antibodies were detected by the addition of fluorescein-labeled goat anti-human IgG or IgM conjugates. Anti-SARS-CoV–positive and anti-SARS-CoV–negative control samples were simultaneously performed. The results were read by an experienced medical technician under the fluorescence microscope. The presence of fluorescence with 10× dilution of serum was considered positive.

Western Blot Analysis of Angiotensin-Converting Enzyme-2

To determine whether inherited differences of the SARS-CoV spike protein receptor angiotensin-converting enzyme (ACE)-2 exist among HCWs, the expression of ACE-2 in peripheral blood mononuclear cells (PBMCs) were examined by Western immunoblotting. Peripheral blood was obtained with ethylenediamine tetra-acetic acid, and platelet-rich plasma was removed after centrifugation at 1,500 revolutions per minute for 10 min. The remainder of blood was then mixed with 3 mL of phosphate-buffered saline solution, overlayed on Ficoll-Paque Plus (Amersham Pharmacia Biotech; NY), and centrifuged at 1,500 revolutions per min for 30 min. Mononuclear cells were isolated from the interface and washed twice with phosphate-buffered saline solution. To examine the effect of fever on the expression of ACE-2, total PBMCs were equally divided and separately cultured at 37°C and 40°C for 24 h. Cells were then lysed in radioimmunoprecipitation buffer with 50 mmol/L Tris-HCl/150 mmol/L NaCl/1% Triton X-100/1% sodium deoxycholate/0.1% sodium dodecylsulfate/protease inhibitor cocktails (Roche; Germany). The protein lysate was centrifuged at 13,500 revolutions/min for 10 min at 4°C, and supernatants were collected and kept at −20°C. Protein concentrations were determined by assay (Bio-Rad DC protein assay kit; Bio-Rad; Hercules, CA). Thirty micrograms of the total protein were added in sample buffer and boiled for 5 min. Protein lysates were then resolved by 7.5% sodium dodecylsulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membrane. After blocking with 5% dry skimmed milk in Tris-buffered saline solution with 0.05% Tween-20 at room temperature for 1 h, membranes were probed with ACE-2 antibodies (R&D; Minneapolis, MN) at 4°C overnight. Membranes were then incubated with secondary alkaline phosphatase-conjugated goat anti-mouse (or rabbit) IgG at room temperature for 1 h. After washing with Tris-buffered saline solution with 0.1% Tween-20, protein bands were visualized (CDP-star; Roche) according to the instructions of the manufacturer.

ACE-2 Binding Assay by Flow Cytometry

Purified PBMCs were harvested and washed twice with fluorescence-activated cell sorter staining/washing buffer. A total of 2 to 5×10⁶ cells were incubated with 1 μg of recombinant spike-1, 2, 3-Fc protein (a gift from Dr. Shie-Liang Hsieh; Yang-Ming University, Taiwan) or IgG1 as isotypic control for 30 min at 4°C. Cells were washed twice and stained with the retinal pigment epithelium-
conjugated anti-human Ig antibodies for 30 min at 4°C. After washing, cells were fixed with fixation buffer for 30 min at 4°C; finally, the fluorescence was detected by fluorescence-activated cell sorter Calibur. ACE-2 binding assays were also performed for PBMCs incubated at 37°C and 40°C for 24 h.

Results

Detection of SARS-CoV in HCWs

All 230 HCWs were ethnic Taiwanese. The male-to-female ratio was 1 to 11. The mean age was 32 ± 8 years (± SD). Thirty-eight of 203 RT-PCR–negative HCWs (18.7%) and 2 of 27 RT-PCR–positive HCWs (7.4%) had underlying diseases (Table 1). Among 230 HCWs, 203 HCWs including 13 non–first-line HCWs showed negative results for both nested RT-PCR assays (Table 2). Two HCWs presented persistent fever (>38.5°C) and subsequently acquired clinical SARS. They were found to have positive results for both nested RT-PCR tests, with 16,900 ± 7,920 copies of RNA per milliliter in the NPS, and they acquired SARS-CoV–specific antibodies. The remaining 25 HCWs were negative for the first nested RT-PCR test but positive for the second nested RT-PCR test. Quantitative RT-PCR tests showed significantly lower titers of viral RNA in the asymptomatic HCWs (312 ± 204 copies per milliliter) or mildly symptomatic HCWs (386 ± 203 copies per milliliter) as compared to those of two HCWs with SARS disease (16,900 ± 7,920 copies per milliliter, p < 0.01). Six of these 25 HCWs presented with mild symptoms, including 2 HCWs with mild fever (body temperature >37°C to <38°C), 5 HCWs with cough, and 1 HCW with diarrhea. Regardless of asymptomatic or mildly symptomatic status, their NPS titers of SARS-CoV were not significantly different, and none had antibodies developing against SARS-CoV. However, 22 of 203 RT-PCR–negative HCWs also had mild symptoms, including 18 HCWs with sore throat or runny nose, 6 with mild fever, 12 with cough, and 3 with diarrhea.

To detect the possibility of contamination in the nested RT-PCR procedure, positive and negative RT-PCR control samples were included in each run. The negative results of the first and second nested RT-PCR tests presented by all of 13 non–first-line HCWs who had no contact history with SARS also support the absence of false-positivity (Table 2). To confirm the specificity of nested RT-PCR, the positive amplified products obtained through agarose gel electrophoretic separation was sequenced and 100% homology was noted as compared with the reference sequence of SARS-CoV reported by Rota et al.8 The sets of primers of the second nested RT-PCR, F2/R1 and F3/R2, were more SARS specific than IN-6/IN-7 and F2/R1 used in the first nested RT-PCR. The corresponding quantitative RT-PCR results were in concordance with a higher sensitivity of the second nested RT-PCR test.

Serologic Tests

With the appropriate convalescent serum samples collected and tested, all of 203 HCWs with negative RT-PCR findings had no detectable IgG or IgM antibodies against SARS-CoV. Two HCWs with SARS disease had detectable antibodies of IgG and IgM classes in their convalescent serum. In spite of positive results by the second nested RT-PCR and low titers of SARS-CoV by quantitative RT-PCR, the remaining 25 HCWs had no detectable SARS-CoV–specific antibodies by either EIA or IIFT (Table 3).

ACE-2 Expression and Function

Although 27 HCWs were found to have at least one positive nested RT-PCR result for SARS-CoV in their NPS, only 2 HCWs with high titers of viral load and persistent fever acquired SARS disease. The above observation that higher titers of virus may preferentially increase the likelihood of developing disease raises the possibility of inherited differences in the ACE-2 expression and function among those subjects. Another possibility that ACE-2 might be inducible by febrile condition was studied by Western immunoblotting and flow cytometry examination of PBMCs at different temperatures. As Figure 2 shows, ACE-2 expression of PBMCs was similar among all subjects either at 37°C or 40°C. A functional study by a binding assay showed that PBMCs did not bind the recombinant spike-1, 2, 3-Fc protein, even after cultured at 40°C for 24 h. These data indicated that the likelihood of developing disease in

Table 1—Underlying Diseases of RT-PCR-positive and RT-PCR-negative HCWs*

| Diseases                  | RT-PCR Negative (n = 38 of 203) | RT-PCR positive (n = 2 of 27) |
|---------------------------|---------------------------------|------------------------------|
| Asthma                    | 5                               | 1                            |
| Hepatitis B               | 8                               | 1                            |
| Diabetes mellitus         | 5                               | 0                            |
| Hypertension              | 0                               | 0                            |
| Hyperlipidemia            | 4                               | 0                            |
| Cerebrovascular disease   | 1                               | 0                            |
| Pituitary tumor           | 1                               | 0                            |
| Depressive disorder       | 1                               | 0                            |
| Hyperthyroidism           | 2                               | 0                            |
| Hepatitis C               | 2                               | 0                            |
| Pulmonary tuberculosis    | 1                               | 0                            |
| Uremia                    | 1                               | 0                            |
| Anemia                    | 1                               | 0                            |

*Data are presented as No.
our subjects was not determined by the expression or function of ACE-2.

**Discussion**

During the severe outbreaks of SARS such as in Hong Kong and Hanoi, 46% and 63% of cases, respectively, were reported among HCWs.12 Despite stringent protective measures according to the World Health Organization guidelines, several hospital outbreaks continued to threaten HCWs throughout Taiwan. Facing the uncertainty of its transmission pattern at that time, we hoped to detect individuals with infection early during the incubation period. Thus, Mackay Memorial Hospital practiced additional safety measures, including centralized accommodation for first-line HCWs and regular NPS screening for SARS-CoV. This extra precaution seemed to block successfully further contagion by isolating 27 individuals who might possibly become transmission sources—in particular, those 2 individuals with significantly higher titers of virus. Although all 25 infected individuals with positive results of the second nested RT-PCR only had negative findings on the follow-up NPS screen and did not acquire SARS, it is possible that these individuals could still have been contagious while carrying low amounts of the virus. In contrast, the individuals who had higher titers of the virus were more likely to acquire clinical SARS and demonstrate a persistent high fever. This suggests that regular NPS screening for SARS-CoV may be more practical in conjunction with daily recording of body temperature.

During the entire period of SARS outbreak in Taiwan, NPS viral loads in non-HCW probable cases admitted in our hospital were approximately 43,460 ± 32,460 copies of RNA per milliliter (unpublished data). For the vast majority of symptomatic SARS patients, it is adequate to use the first nested RT-PCR. However, the second nested RT-PCR is more sensitive for detection of SARS-CoV in low copy numbers (< 800 copies of RNA per milliliter), as evidenced by the corresponding quantitative RT-PCR. During the early screening period, the positive results of the second nested RT-PCR detected in a significant number of HCWs were really worrisome to us. A false-positivity of the nested RT-PCR was unlikely because negative and positive control samples were also simultaneously performed for every run. Retrospectively, the negative results of both nested RT-PCR tests presented by all of 13 non–first-line HCWs who had no contact history with SARS also support the absence of false-positivity. The specificity of nested RT-PCR was confirmed by sequencing the positive amplified products and compared with the reference sequence of SARS-CoV. These HCWs were required to wear N-95 respirators and stay isolated at home or a centralized accommodation for 3 days. Fortunately, the second

### Table 2—Comparison of Two Nested RT-PCR Results With Corresponding Viral Loads of SARS-CoV in the NPS Among HCWs

| Groups of HCWs                  | Subjects, No. | First Nested RT-PCR | Second Nested RT-PCR | Viral RNA, Copies per Milliliter, mean ± SD | Contact History With SARS |
|---------------------------------|---------------|---------------------|----------------------|--------------------------------------------|---------------------------|
| Negative nested RT-PCR          | 203           | Negative            | Negative             | 0                                          | Yes (n = 190); no (n = 13) |
| Positive nested RT-PCR          |               |                     |                      |                                            |                           |
| Asymptomatic                    | 19            | Negative            | Positive             | 312 ± 204                                  | Yes                       |
| Mildly symptomatic              | 6             | Negative            | Positive             | 386 ± 203                                  | Yes                       |
| SARS*                           | 2             | Positive            | Positive             | 16,900 ± 7,920                             | Yes                       |

*p < 0.01 compared to mildly symptomatic and asymptomatic HCWs.

### Table 3—Comparison of Serum Antibodies Specific for SARS-CoV Among HCWs

| Group of HCWs                  | Subjects, No. | First Serum | Second Serum |
|--------------------------------|---------------|-------------|--------------|
|                                |               | EIA         | IIFT         | EIA         | IIFT         |
|                                |               | Ig IgG IgM | Ig IgG IgM   | Ig IgG IgM |
| Negative nested RT-PCR         | 203           | Negative    | Negative     | Negative    | Negative     |
| Positive nested RT-PCR         |               | Negative    | Negative     | Negative    | Negative     |
| Asymptomatic                   | 19            | Negative    | Negative     | Negative    | Negative     |
| Mildly symptomatic             | 6             | Negative    | Negative     | Negative    | Negative     |
| SARS*                          | 2             | Positive    | Positive     | Positive    | Negative     |

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nested RT-PCR turned negative for all of them in the second or third follow-up NPS.

Those HCWs who were either mildly symptomatic or had low titers of virus in NPS screening did not become SARS patients by clinical criteria. This experience was somewhat different from the study reported by Ho et al\textsuperscript{5} in Singapore. In their study, although no asymptomatic HCW was found by serologic testing to have SARS-associated coronavirus infection, antibodies to SARS-CoV developed in 3.2\% of mildly symptomatic HCWs. By contrast, Chow et al\textsuperscript{6} showed that HCWs with positive neutralizing antibodies had symptoms indicative of SARS. None of the HCWs with nonspecific symptoms showed serologic evidence of subclinical infection. SARS demonstrates a spectrum of infection, hence the detection of low-titer colonization of SARS-CoV without seroconversion in asymptomatic and mildly symptomatic HCWs is to be expected. The findings in our study confirm that a low-level transmission of virus is less likely to trigger epidemics, as previously suggested by Peiris et al\textsuperscript{7}.

Our previous data also revealed that 63 of the 346 reported SARS cases (10.7\%) in Taiwan did not have appropriate seroconversion despite having a positive RT-PCR result (Mei-Shang Ho; unpublished data). It is possible that these HCWs had been exposed to the amounts of SARS-CoV that were not adequate to elicit an effective immune response, similar to the situation occurring after vaccination.\textsuperscript{15} This is the first time for such a finding that by using a more sensitive technique, we have found that HCWs exposed to patients with SARS may have colonization without seroconversion. In other words, it suggests that the virus is not replicating within our tissue since no IgG or IgM antibody response is detected. The detection of subclinical viral colonization as evidenced in the NPS specimens that were collected prospectively and assayed with a more sensitive nested RT-PCR procedure is an important finding, as a result of the implemented of our stringent hospital policy. Why the small amount of SARS-CoV detected by NPS remained temporarily in some HCWs and failed to invade the host might be related to several factors discussed below.

Although SARS is a seemingly fatal virus, there has been a wide range of reactions, from mild symptoms to a disseminating syndrome. Although the mechanism of active viral disease developing from the colonized status is not fully understood, pathogenic factors such as the initial viral load may play an important role as suggested by our results. First, host factors need be considered. It has been reported that total secretory IgA is significantly associated with reduction of symptoms; total protein in nasal washings has been suggested to protect against infection.

Figure 2. Western immunoblotting of ACE-2, the SARS-CoV spike protein receptors, in PBMC from HCWs. The basal expression of ACE-2 was not different between HCWs with or without SARS-CoV infection. The expression of ACE-2 was not affected by culturing in 40°C for 24 h. RT(-) = negative for both nested RT-PCRs; RT(+) = positive for only the second nested RT-PCR, SARS = HCWs with SARS; actin was used as an internal control. Vero-E6 = positive control.
and may be associated with resistance in human coronavirus 229E infection.\textsuperscript{16} Genetic predisposition such as specific human leukocyte antigen types has been shown to contribute to the different clinical outcomes following SARS-CoV infection.\textsuperscript{17}

Secondly, the expression and function of ACE-2 may also help to explain the spectrum of SARS reactions. In November 2003, this metalloprotease was identified as the functional receptor for SARS-CoV in mediating cell entry and fusion.\textsuperscript{18} The selection of PBMCs in our study was based on availability and the inaccessibility to collect a large amount of other cell types that possess high levels of ACE-2. Even though PBMCs have been considered as devoid of ACE-2,\textsuperscript{19} we have shown that ACE-2 expression can be detected using Western immunoblotting. In addition to examining the basal expression of ACE-2 from PBMCs, we have found that the expression and function of ACE-2 in PBMCs were not inducible by culturing at the higher temperature, suggesting that fever may not increase the likelihood of developing SARS. Our results did not demonstrate any significant difference in the ACE-2 expression and function of the subjects, but these results may not necessarily apply to other cell types. Due to the difference of ACE-2 distribution in various tissues and the inability to show a positive binding assay, we cannot conclude its exact role in the severity of SARS. Further studies are needed to draw firmer conclusions about the relationship of host factors to the outcome of SARS.

In conclusion, utilizing a more sensitive detection technique, we are able to show that 25 of 217 well-protected HCWs (11.5\%) exposed to patients with SARS may have colonization without demonstration of specific antibodies to the virus. Only those with significant clinical symptoms or disease acquire active immunity. Thus, regular NPS screening for nested RT-PCR testing in conjunction with a daily recording of body temperature in all first-line HCWs may provide an effective way of early detection. Although all HCWs detected by the second nested RT-PCR did not acquire SARS, there is no assurance whether these individuals carrying low amounts of the virus were capable of transmitting the virus to susceptible individuals. We would recommend a conservative infection control program to require nested RT-PCR-positive HCWs to wear N-95 respirators and stay isolated at home or a centralized accommodation for 3 days.

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