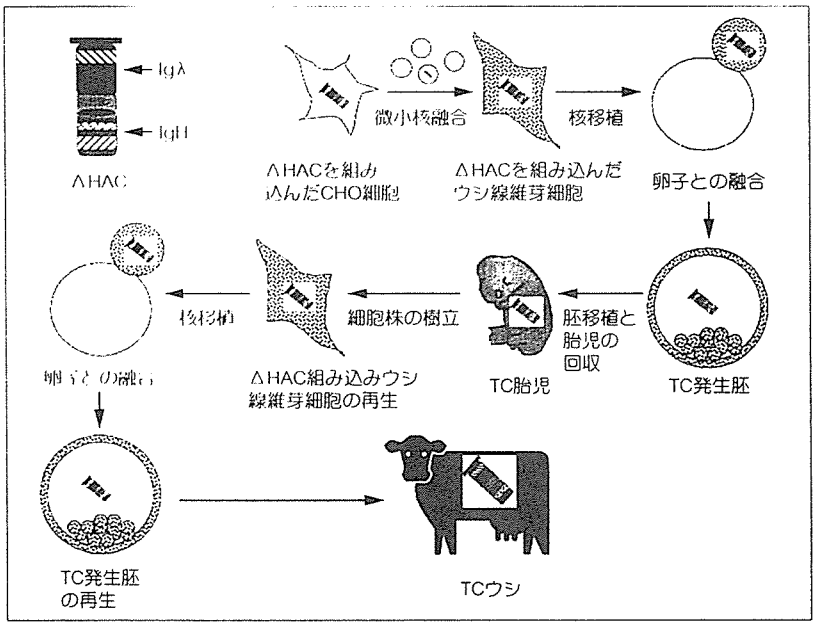

図2 TCウシ作製法

(左上部) それぞれIgλ座位およびIgH座位を含むヒト第22番染色体(黒)およびヒト第14番染色体(赤)からなるヒト人工染色体(HAC)の構造。このHACをCHO (Chinese hamster ovary) 細胞からウシ線維芽細胞に微小核融合法で導入し，核を除去した卵子(黄色)と融合させ，胚へと発生させる。得られた胚を代理母ウシへ移植して，妊娠60日でTC胎児を回収し，この胎児からTC線維芽細胞を再調整する。得られた再構築TC線維芽細胞に同様の操作を繰り返してさらに形質転換操作を進める。 (文献5より改変)

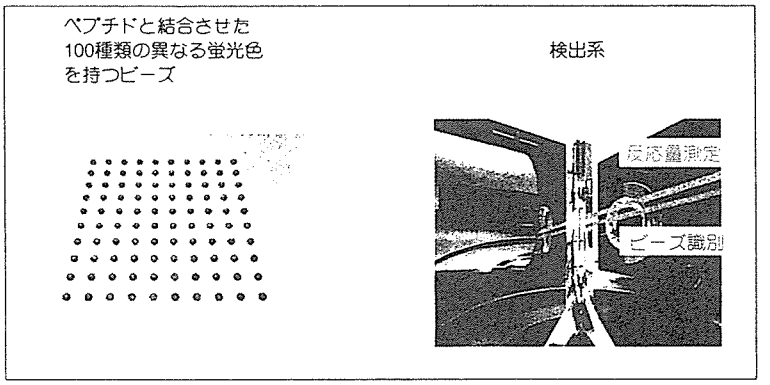

図3 Luminexシステムによるペプチド抗体の解析

希釈した血清検体を100種類の異なる蛍光色を持ち，かつ各ペプチドを結合させたビーズと反応させた後，フローメトリーにより各ペプチドに結合したIgG量を測定した。

の多くは医療従事者であったので，SARS患者と接触したもののSARSを発症しなかった接触者群(230名)の他に，医療従事者群(50名)を対照とした。Luminexを用いた解析の結果，42のペプチドのうちspike由来のS791，membrane由来のM207およびnucleocapsid由来のN161

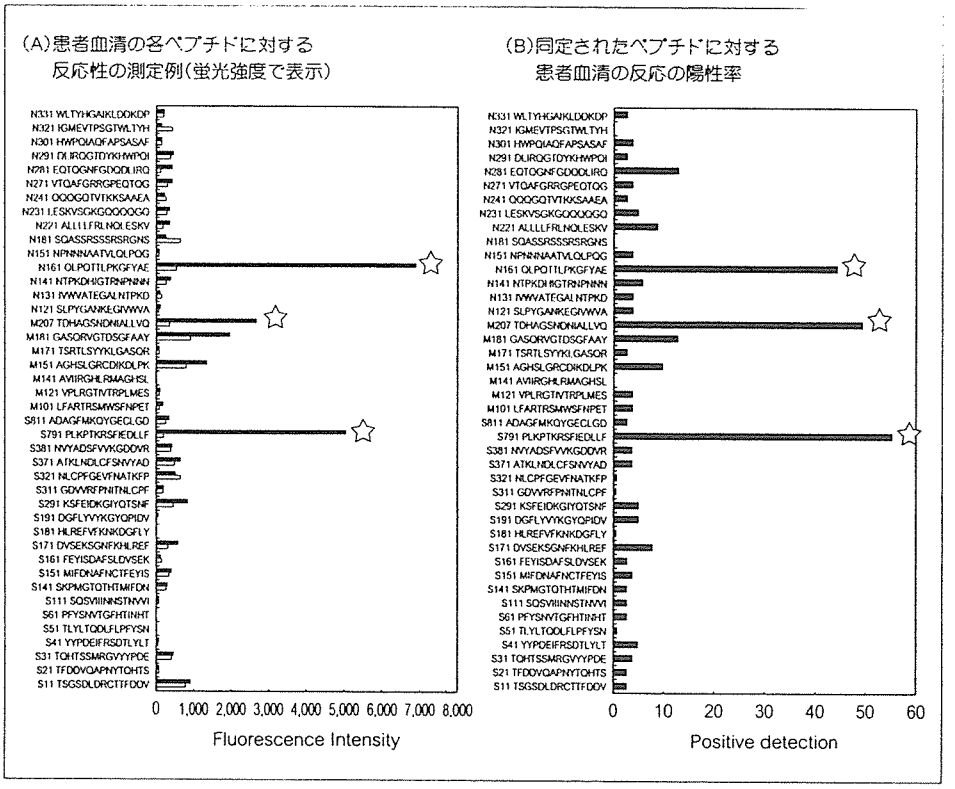

図4　SARS 患者回復期血清の SARS-CoV 由来ペプチドライブラリとの反応性

図3に示した A) 患者血清（黒）または対照者（白）とペプチドの反応性（蛍光強度で表示）の平均値。B) 同定されたペプチドと反応する SARS 患者回復期血清の陽性率。

（文献6より改変）

表1　SARS 患者血清で同定されたの3種のエピトープ

	SARS 患者	SARS 患者以外	
		接触者	医療従事者
S791	51	8	8
M207	60	4	6
N161	42	9	2

数値は患者群あたりの陽性率（%）

と名付けた3本のペプチドについて特に患者血清で陽性率が高いことが明らかとなった（図4，表1）。このことは SARS 患者では実際に SARS-CoV の蛋白質抗原に対する抗体価が上昇しており，それらの蛋白質が SARS-CoV 中和ヒト抗体作成のための標的となることを示す。

V　組み換え SARS-CoV 蛋白質抗原の作成

ヒト抗体産生動物に SARS-CoV 中和活性を持つ抗体を産生させるための免疫方法は多岐にわたる。すなわち，SARS-CoV 本体を不活化するなどして投与する方法，特定の SARS-CoV 因子を組

み込んだDNAワクチンを投与する方法，改変ウイルスベクターにSARS-CoV因子を組み込み生ワクチンとして使用する方法，およびSARS-CoVの組み換え蛋白質抗原を使用する方法などが考えられる。これらの方法はそれぞれメリット・デメリットがあるが今回我々はSARS-CoVの組み換え蛋白質抗原を使用する方法を選択した。現在では病原性の高い病原体の取り扱いは法的整備も進んでおり，それらの輸送，大量培養の実施，またこれら病原体因子を持つ組み換え生物の作出にはその安全性を担保するためにクリアしなければならないハードルが非常に多い。そのため国外で発生した新興感染症などに対応するための病原体本体の確保や，その抗原の入手は事実上不可能な場合がある。これに対し組み換え蛋白質での抗原作成は，得られる蛋白質自体には通常病原性がなく安全に取り扱える上，病原体本体を入手しなくとも遺伝情報のみをもとに実施可能であり，他の方法に比べて抗原入手までの期間が短縮できる可能性が高い。

組み換え蛋白質作成系は，無細胞系，大腸菌などの原核細胞系，昆虫細胞系，および培養細胞系などに大別される。今回我々は大腸菌の系を選択することとした。良く知られているように大腸菌で作成した組み換え蛋白質は，真核細胞由来の蛋白質やウイルス蛋白質には存在し，またそれらが抗原性に大きく影響すると考えられている糖鎖修飾を受けていない。このことはヒト抗体産生動物免疫のための抗原としての不利な点である。その一方で大腸菌蛋白質発現系はこれまでに科学的知見が蓄積されており，当該蛋白質を発現させること，発現量を確保すること，さらに得られた蛋白質を精製すること，が他の系に比べて圧倒的に容易である。また今回の研究では最終的な免疫動物がウシであり，1回の免疫にはmg単位で蛋白質が必要であると予想されたため，最終的には100 mg単位での組み換え蛋白質を確保する必要があった。このため，今回の研究では大腸菌での発現系を選択した。また精製を容易にするため高濃度の変性剤存在下でもアフィニティ精製が可能なヒスチジンタグ融合蛋白質として標的蛋白質を発現させることとした。作成する組み換え蛋白質は前述のベトナムSARS患者血清の解析でエピトープとなりえることが判明したspike, nucleocapsidおよびmembraneの各蛋白質をもとにデザインすることにした。これまでの検討の結果，デザインした組み換え蛋白質を大量に発現させ，さらに純度95%以上まで均一に精製することに成功している。

組み換え蛋白質作成過程で問題となったのがヒスチジンタグの存在である。ヒスチジンタグは組み換え蛋白質の精製で繁用されており，将来的にヒスチジンタグを利用して精製された組み換え蛋白質が様々な局面で応用されると考えられる。このことはヒスチジンタグを持つ蛋白質をそのまま抗体作成に使用した場合，標的蛋白質本体とは無関係の交差反応を惹起する可能性を示唆する。このため本研究ではヒスチジンタグの除去は必須であった。そこで各組み換え蛋白質は酵素処理によりヒスチジンタグを除去可能なデザインとした。さらに大量の組み換え蛋白質から効率よくヒスチジンタグを除去できるように筆者らが新たに開発したプロトコール(特許申請中)にて，現在ではヒスチジンタグなしの免疫用組み換え蛋白質抗原の最終標品を100 mg単位で調整可能となっている。これらの抗原は，ヒト抗体産生動物で免疫実験に供され，得られた抗体のウイルス中和活性を解析中である。

Ⅵ SARS-CoV感染症の治療薬および免疫療法

SARS流行時には対症療法的に種々の抗ウイルス薬が患者に投与されたが明瞭な効果を示す薬物は同定されなかった。その後SARS-CoVがSARSの病原体であることが明らかとなり，抗SARS-CoV薬の探索が盛んに行われている。分子創薬の観点からはSARS-CoVの3CLプロテアーゼやポリメラーゼの阻害物質探索が行われており，培養細胞ベースの活性測定ではSARS-CoVの増殖を抑制するものも報告されている。またspike蛋白質による宿主細胞との結合とその後のウイルス粒子融合を阻害するための薬物探索も行われており，ペプチドベースの試みも行われている。特に着目されるのはRNA干渉を利用したSARS-CoV

の抑制実験で，サルを用いた動物実験で予防・治療の両面で有効であるとの報告がある。

しかしながら動物実験でその有効性が確認されている例はむしろワクチンや受動免疫を利用した免疫療法について報告が多い。SARS-CoV に対する DNA ワクチン，同ウイルスの組み換え蛋白質などで免疫したマウスから得た抗血清によるマウスの受動免疫によるウイルス感染防御が報告されている。さらに SARS 患者より樹立したヒト B 細胞ハイブリドーマや SARS-CoV の spike 蛋白質で免疫したヒト抗体産生マウスより作成したモノクローナル抗体でも，動物実験で受動免疫の有効性が報告されている。これらの知見は SARS-CoV 中和ヒトポリクローナル抗体開発の意義を支持するものと考えられる。

Ⅶ おわりに

本研究の目的は SARS-CoV に対応するヒト抗体医薬品を開発することである。この開発研究を成功させることで，新興・再興感染症などに迅速に対応するための抗体医薬を提供するための一つのプロトタイプを提供できるであろうと確信している。さらに高力価ポリクローナル中和抗体が感染症分野の主要な医薬品になるための先駆けとなることを期待している。

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Original Article

"Syndromic Surveillance within a Hospital" for the Early Detection of a Nosocomial Outbreak of Acute Respiratory Infection

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SUMMARY: We have performed intra-hospital syndromic surveillance to rapidly detect nosocomial acute respiratory infection outbreaks in both inpatients and health care workers in a hospital. Syndromic surveillance allows the rapid detection of sudden outbreaks, including infections caused by unknown pathogens. This approach depends on the identification of specific "symptoms" as signs of a possible outbreak, with no need for specific diagnoses. Moreover, syndromic surveillance is quick, easy, and inexpensive. Nosocomial infection surveillance is usually performed on inpatients only. However, during the outbreaks of SARS and seasonal influenza, for example, many hospital personnel were infected. In cases of this kind, in order to quickly detect the prevalence of such infections, a surveillance system that includes hospital personnel is essential. This surveillance is promising as a strategy to prepare for re-outbreaks of SARS and the emergence of novel influenza pandemics.

INTRODUCTION

Severe acute respiratory syndrome (SARS) emerged from 2002 to 2003. According to the World Health Organization (WHO), over 8,000 infected patients were reported during this period. A notable problem of SARS is the number of health care workers infected: at 1,706 persons, such cases accounted for 21% of all reported cases (1).

For the early detection of hospital outbreaks of acute respiratory infections (ARIs) that develop with a short incubation period and can spread by airway droplet transmission, such as SARS (2) and influenza (3,4), the surveillance method should be simple and rapid, and hospital personnel should be included in the surveillance.

We therefore applied the strategy of syndromic surveillance – a method based on surveillance for symptoms only, which does not depend on conclusive diagnoses or laboratory results (5). The system applied not only to inpatients but also hospital personnel.

Our objective was to assess and validate the usefulness of syndromic surveillance that includes hospital personnel to detect the outbreak of ARIs in our hospital.

MATERIALS AND METHODS

In Japan, ARIs are most prevalent in winter (from November to March), and this study was therefore performed during winter months. Basically, the first season of the period of study was from 2003 to 2004, the second season was from 2004 to 2005, and the third season was from 2005 to 2006.

Subjects showing both a fever of >38°C and respiratory symptoms were rated as having symptoms of ARIs and were included in the surveillance. Respiratory symptoms included upper airway symptoms (nasal discharge and sore throat), lower airway symptoms (cough, sputum, dyspnea, and reduced SpO2), or chest X-ray evidence of pneumonia. For each reported case with respiratory symptoms, the Infection Control Team (ICT) recommended a rapid test for influenza.

Subjects consisted of all patients hospitalized at the International Medical Center of Japan (IMCJ) hospital during the above-mentioned periods, in addition to the nurses, nursing assistants, physicians, laboratory technicians, pharmacists, administrative personnel, and students. If a case consistent meeting the definition of an ARI was identified, the head of each section immediately filled out a surveillance sheet and submitted it to an ICT. The ICT visited each ward every day, and had an interview with the head nurse, asking whether there were cases showing the target syndrome, and collected report papers. Report papers submitted on holidays were collected by the ICT on the next day, and each week after the collection, the results of the reports were documented on the hospital intranet for the hospital personnel. The IMCJ is a general hospital that is located in Tokyo. It has 925 beds and 28 health-care units. It also has 155 physicians, 585 nurses, 100 laboratory technicians and pharmacists, and 80 administrative personnel.

This study was approved by institutional review boards and the infection control committee of the IMCJ.

RESULTS

The numbers of cases that showed acute respiratory symptoms in each season are shown in Figure 1.

During the 106-day period from December 17, 2003 to March 31, 2004 (first season), 215 cases were reported. Rapid tests for influenza were performed on 109 individuals (51%), of whom 49 were rated as positive (the positivity rate was 23% of the total reported cases, and 45% of the rapid-tested cases). All positive cases were type A influenza. The surveillance period of the 1st season was comparatively short, because surveillance was performed as a provisional trial in this season. Reported cases included 168 inpatients (78%),

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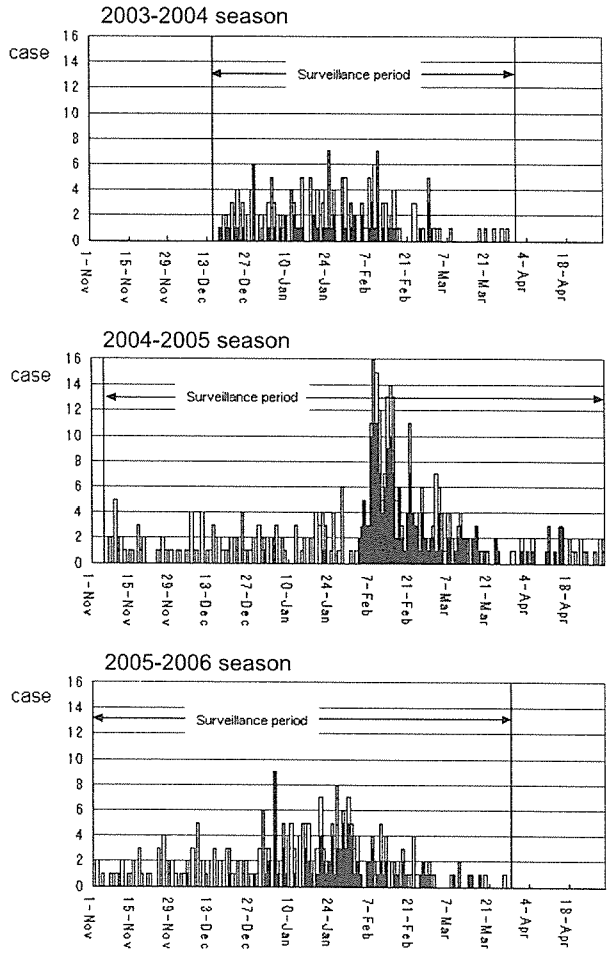

Fig. 1. Number of cases that showed acute respiratory symptoms in the hospital. Closed bar, rapid test influenza positive.

25 nurses (12%), 14 physicians (7%), and 4 technologists (2%). The outcome of this provisional trial has already been reported (6).

During the 175-day period from November 7, 2004 to April 30, 2005 (the second season), 382 cases were reported. An obvious outbreak of acute respiratory symptom cases was observed in early February. Rapid tests for influenza were performed on 261 individuals (68%), of whom 169 were rated as positive (the positivity rate was 44% of the total reported cases, and 65% of the rapid-tested cases). This peak consisted of an influenza outbreak at our hospital. Influenza was rated as type B in 130 cases (77%) and type A in the remaining cases. Reported cases included 268 inpatients (70%), 68 nurses (18%), 29 physicians (8%), 8 technologists (2%), and 8 administrative personnel (2%). Cases reported during this period were classified into inpatients and hospital personnel, and their courses are shown in Figure 2. Reports from inpatients constituted the majority of reports during the non-epidemic period, while reports from hospital personnel accounted for about 50% of reports during the influenza epidemic period.

During the 151-day period from November 1, 2005 to March 31, 2006 (the third season), 270 cases were reported. Rapid tests for influenza were performed on 204 individuals (76%), of whom 89 were rated as positive (the positivity rate was 33% of the total reported cases, and 44% of the

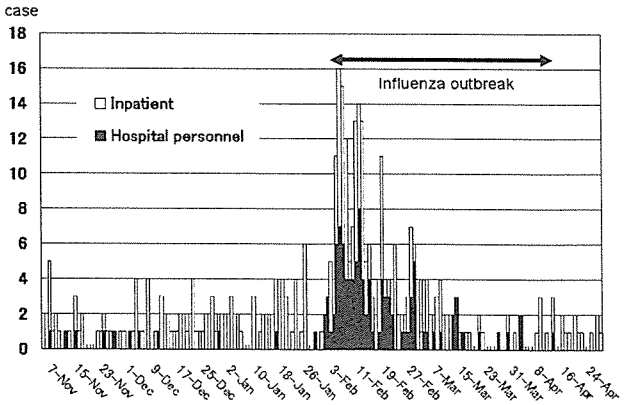

Fig. 2. Number of cases with respiratory symptoms defined by patient background. November 7, 2004 - April 30, 2005.

rapid-tested cases). Eighty-eight cases (99%) were type A influenza. Reported cases included 215 inpatients (80%), 35 nurses (13%), 8 physicians (3%), and 6 technologists (2%).

When cases showing respiratory symptoms or many cases of influenza were observed, the ICT immediately performed an intervention and took measures such as the isolation of patients, strengthening of anti-infection measures, and recommendation of rest to hospital personnel with infection.

DISCUSSION

Usual nosocomial infection surveillance can be divided into two types: "hospital-wide surveillance" and "targeted surveillance" (7). Hospital-wide surveillance involves all patients managed at a given hospital. Its advantage lies in the fact that detection of the outbreak of nosocomial infection is easier with this type of surveillance. Its disadvantages are the amount of labor needed, low efficiency, and difficulty in comparing the results with those from surveillance at other hospitals. The second type, or targeted surveillance, has the advantage of being generally effective and allowing easy comparison with the results of other hospitals. Targeted surveillance focuses on surgical-site, bloodstream, and urinary tract infection, and ventilator-associated pneumonia. However, targeted surveillance is not suitable for the early detection of diseases such as influenza and SARS, which tend to show sudden major outbreaks. Therefore, we need a much easier method of "hospital-wide surveillance" to detect these types of infection outbreaks. In the present study, we focused on the strategy of "syndromic surveillance" (5). Syndromic surveillance allows the rapid detection of sudden outbreaks, including infection caused by unknown pathogens. This approach depends on the identification of specific "symptoms" as signs of a possible outbreak, with no need for specific diagnoses. In recent years, this method has been used for the early detection of bioterrorism. Moreover, syndromic surveillance is quick, easy, and inexpensive.

The surveillance allowed the precise detection of outbreaks of influenza within our hospital during the survey periods. In particular, during the second season of the study, sudden outbreaks of influenza within our hospital were clearly documented. The peak of the outbreak in the hospital coincided with the peak of influenza prevalence in the whole of Japan reported by the National Institute of Infectious Diseases, Japan (8). However, further investigation is needed to fully elucidate the sensitivity and specificity.

Nosocomial infection surveillance is usually performed on inpatients only. However, during the outbreaks of SARS in 2003, many hospital personnel were infected, as previously mentioned (1,2). In cases of this kind, in order to quickly detect the prevalence of such infections, a surveillance system that includes hospital personnel is essential. The WHO has proposed a strategy called "SARS Alert" in preparation for a recurrence of the disease (9). This is a judgment standard stipulating that "if two or more health care workers have clinical evidence of SARS in the same health-care unit and with onset of illness in the same 10-day period, a recurrence of SARS must be suspected". In order to detect cases that are consistent with the SARS Alert, a symptomatic surveillance that includes hospital personnel must be performed. Apart from SARS, there are many other ARIs that may involve hospital personnel, such as influenza, respiratory syncytial (RS) virus, and others. Even in the case of a major outbreak of a novel influenza virus, which is widely feared to be possible in the near future, a method of syndromic surveillance that includes hospital personnel will be effective. We were able to obtain clear information about infection among hospital personnel through the surveillance. For example, a significantly large number of personnel cases were reported during the influenza season (Figure 2). Highly infectious diseases such as influenza also induce outbreaks involving hospital personnel. Hospital personnel with respiratory symptoms immediately put on masks (10), and those who were found to have influenza were instructed to undergo treatment at home. As a result of these measures, it was possible both to control nosocomial infection and, simultaneously, implement treatment among hospital personnel who had fallen ill.

Finally, the problems associated with this method require some discussion. This surveillance adopted a method by which hospital personnel who detected a symptomatic case filled in a case report form and submitted it to the ICT. With this method, the cooperation of hospital personnel is indispensable. To gain adequate cooperation of personnel, it is necessary to provide information and an explanation about the planned surveillance to hospital staff. Since this method depends on "reporting" from the place of clinical practice, there is a possibility that the number of reported cases decreases with a decrease in the sense of impending crisis in physicians and nurses, which was suggested by the definite decrease in the number of reported cases after an influenza epidemic compared with the number before the epidemic in all 3 seasons (Figure 1). Ideally, this surveillance would be performed throughout the year. However, surveillance only during high-risk periods may be more practical. In addition, a prerequisite condition is the effective functioning of the teams responsible for initiating the anti-infection measures, such as the ICT.

We have proposed syndromic surveillance as a method for the early detection of outbreaks of ARIs in hospitals. This method is simple and quick, and can be performed by any hospital. It may be applicable to developing countries as well. This surveillance method is promising as a strategy to prepare for re-outbreaks of SARS and the emergence of novel influenza pandemics.

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Short Communication

Trial Surveillance of Cases with Acute Respiratory Symptoms at IMCJ Hospital

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SUMMARY: We have developed a surveillance system that can detect a severe acute respiratory syndrome (SARS) outbreak in a hospital as quickly as possible using the "SARS alert" strategy proposed by the World Health Organization (WHO). Our research examined hospital staff and in-patients during the winter of 2003/2004. We defined patients with a fever of over 38°C and respiratory symptoms as "cases with acute respiratory symptoms." During the study period, 215 such cases (78% in-patients; 22% hospital staff members) were reported. A rapid diagnostic test for influenza was performed on 131 individuals, with 52 having positive results. There were no cases fulfilling the definition of SARS provided by the WHO in their SARS alert. The present surveillance system will be of use in the early detection of a SARS epidemic in a hospital as well as in early detection of similar illnesses accompanied by acute respiratory symptoms, such as influenza.

Severe acute respiratory syndrome (SARS) haunted the world from November 2002 to July 2003. According to the World Health Organization (WHO), over 8,000 infected patients and nearly 800 deaths were reported in 26 regions during this period. An extremely large problem in the case of SARS is the number of health care workers (HCWs) infected; at 1,706 persons, the figure accounted for 21% of all reported cases (1; http://www.who.int/csr/sars/country/table2004_04_21/en/). Because of this problem, the WHO has proposed a new surveillance strategy known as the "SARS alert" (2; http://www.who.int/csr/sars/postoutbreak/en/). If a SARS alert occurs, the WHO recommends that strict infection control procedures be adopted immediately. However, the introduction of this policy requires daily surveillance in accordance with the definition of a SARS alert. Additionally, this surveillance targets not only in-patients but also hospital personnel. To date, the WHO has not yet indicated any specific methods for the application of SARS alert surveillance to hospital personnel.

Therefore, we attempted to create a new surveillance system to detect clinical SARS cases as defined by the SARS alert in both patients and HCWs. To facilitate the detection of SARS as well as other respiratory infectious diseases such as influenza, the present surveillance focused on cases with "acute respiratory symptoms".

These definitions used for this surveillance were a fever of over 38°C and one or more symptoms of respiratory tract illness (RTI), including both upper RTI (rhinorrhea or sore throat) and lower RTI (coughing, sputum, shortness of breath, decreased SpO_2, or radiographic evidence of lung infiltrates consistent with pneumonia or respiratory distress syndrome [RDS]).

The subjects were all in-patients, nurses, doctors, technicians, pharmacists or other medical staff at the International Medical Center of Japan (IMCJ) hospital, Tokyo, Japan. The study period was from December 2003 to March 2004. If a patient or HCW with acute respiratory symptoms was identified, the head of each section filled in a surveillance report and submitted it to an infection control team (ICT). The results of the surveillance were analyzed and released weekly to hospital staff by hospital intranet.

During the study period, 215 cases with acute respiratory symptoms were reported. Their median age was 39.0 years of age (range: 5 mos-99 years of age), and the male:female ratio was 1:1.05. Wards in which numerous cases were reported were the pediatric ward (36 cases), the respiratory ward (20 cases) and the private room ward (18 cases). The identified cases included 168 in-patients (78%), 26 nurses (12%), 15 doctors (7%), 4 technicians (2%) and 2 pharmacists (1%). A rapid test for influenza (Espline®; Fujirebio, Inc., Tokyo, Japan) (3) was performed in 131 cases (61%), and 40% of tested individuals were found to be positive. Trends in the reported cases are shown in Figure 1. There was a peak in the number of reported cases from the 3rd week of January to the 2nd week of February, coinciding with a peak in influenza cases at the IMCJ hospital. Additionally, these peaks coincided with a peak in the nation wide spread of influenza in Japan (4; http://idsc.nih.go.jp/idwr/kanja/weeklygraph/01flu-e.html).

During the surveillance period, one cluster of cases with acute respiratory symptoms was found in our hospital. The episode was observed in the respiratory ward and included 11 cases with acute respiratory symptoms; 4 of which tested positive on the rapid diagnostic test for influenza. This finding caused the ICT to quickly introduce appropriate infection control measures such as cohort isolation, prophylactic use of oseltamivir, and limitations on the admission of new patients. With this intervention, the cluster was quickly controlled.

During the study period, no actual SARS alert cases that met the WHO definition were observed.

SARS is characterized by its high transmissibility to HCWs and becomes widespread via nosocomial infection (5,6). Therefore, both in-patients and HCWs with symptoms must be constantly monitored in order to detect a SARS outbreak

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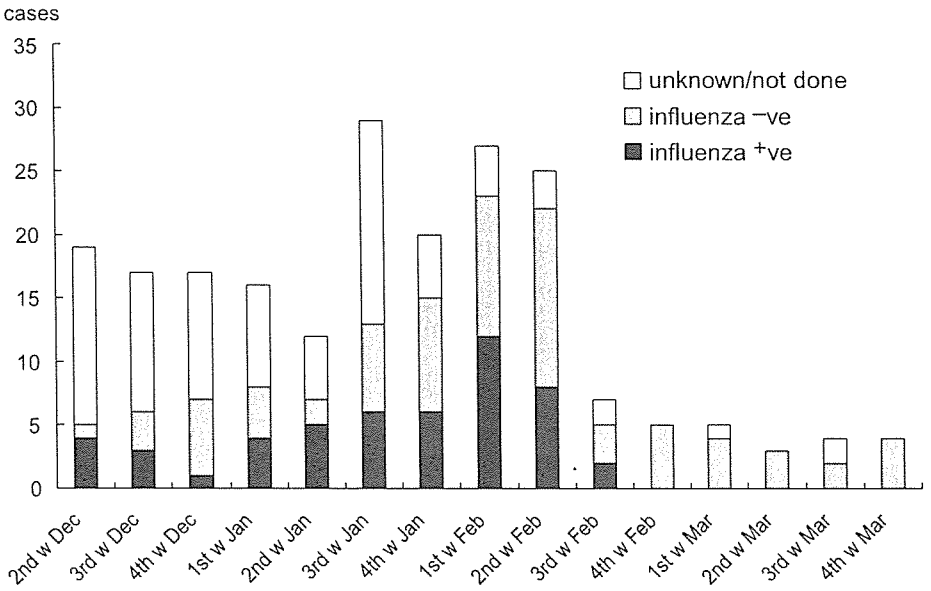

Fig. 1. Trend graph of reported case with acute respiratory symptoms between the 2nd week of December 2003 and the 4th week of March 2004.

in a hospital in the early stages. The SARS alert strategy proposed by the WHO is an operational definition used to ensure that appropriate infection control and public health measures are implemented until SARS has been ruled out as a cause of pneumonia or RDS.

This policy defines SARS cases clinically as cases with a fever of over 38°C, with one or more symptoms of lower RTI (coughing, difficulty breathing, or shortness of breath), with radiographic evidence of lung infiltrates consistent with pneumonia or RDS, and with no alternative diagnosis that can fully explain the illness. SARS alert situation is defined as one or both of the following:

i) two or more HCWs in the same health care unit fulfilling the clinical case definition of SARS and whose onset of illness occurs within the same 10-day period; and
ii) hospital-acquired illness in three or more persons (HCWs and/or other hospital staff and/or patients and/or visitors) in the same health care unit fulfilling the clinical case definition of SARS and whose onset of illness occurs within the same 10-day period.

Because the threat of infection involves not only SARS but also other emerging respiratory virus infections (i.e., new types of influenza), we attempted to create a system that can also detect acute respiratory infections such as influenza in a hospital. Because the early clinical features of SARS and influenza are quite similar, some confusion in clinical settings is expected. Hence, a "syndromic surveillance" system, that is, a system that detects acute respiratory symptoms without regard to the pathogenic virus, must be developed. Therefore, we partially modified the WHO's SARS alert strategy and introduced a new method of surveillance for the early detection of SARS and influenza.

Our criteria for the definition of disease differed from that of the WHO in that it included upper RTI and (ii) it did not require pneumonia findings in chest X-rays. We felt that adding these changes would allow the detection of influenza outbreaks in a hospital as well.

An epidemic of cases with acute respiratory symptoms during the aforementioned period was effectively monitored during surveillance at IMCJ hospital. An outbreak of influenza at the hospital was also detected by the present surveillance system. Information provided by surveillance was effectively used for infection control. Fortunately, there were no cases that met the definition of SARS provided by the WHO in their SARS alert. Hospital staff should be informed as soon as possible about the spread of infectious diseases in the hospital. We used hospital intranet for this purpose, and information was quickly conveyed to the appropriate divisions of the hospital.

The present surveillance strategy will be of use in the early detection of a SARS epidemic in a hospital as well as in the early detection of similar illnesses accompanied by acute respiratory symptoms such as human influenza and new types of influenza. Further study is needed to improve the sensitivity and specificity of this surveillance.

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RAPID AWARENESS AND TRANSMISSION OF SEVERE ACUTE RESPIRATORY SYNDROME IN HANOI FRENCH HOSPITAL, VIETNAM

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Abstract. A case-control study was conducted to examine the relationship between severe acute respiratory syndrome (SARS) and the time-dependent precautionary behaviors taken during an outbreak of SARS in Hanoi French Hospital (HFH), Vietnam. Masks (odds ratio [OR] = 0.3; 95% confidence interval [CI]: 0.1, 0.7) and gowns (OR = 0.2; 95% CI: 0.0, 0.8) appeared to prevent SARS transmission. The proportion of doctors and nurses who undertook each measure significantly improved (χ^2 = 9.8551, P = 0.043) after the onset of secondary cases. The impact of individual behaviors on an outbreak was investigated through mathematical approaches. The reproduction number decreased from 4.1 to 0.7 after notification. The basic reproduction number was estimated, and the use of masks alone was shown to be insufficient in containing an epidemic. Intuitive results obtained by means of stochastic individual-based simulations showed that rapid improvements in behavior and isolation would increase the probability of extinction.

INTRODUCTION

Notwithstanding the announcement of containment by the World Health Organization (WHO) in 2003,[1] severe acute respiratory syndrome (SARS) has remained a matter of concern worldwide, and it is not surprising that several cases of SARS have reemerged, for example, in China in April 2004.[2] Although the mode of transmission remains partially unclear, especially with regard to airborne transmission[3] and superspreading events,[4,5] it appears to occur predominantly by large droplets, direct contact with infectious material, or contact with fomites contaminated with infectious material.[6,7] The most effective containment measures identified to date include the tracing of contacts,[8] quarantine,[9] triage and early case detection,[10,11] and isolation.[12] Further, because the close contact required for transmission easily occurs in hospital settings,[13–15] nosocomial spread was determined as one of the major epidemiologic features of SARS.[7,16,17] The elimination of hospital transmission through enhanced infection control practices is therefore a crucial control measure.

An early study in Hong Kong showed that the practice of droplet and contact precautions was adequate in most clinical settings in significantly reducing the risk of infection after exposure to patients with SARS,[18] and if practiced by a high proportion of susceptible individuals, precautionary measures are expected to significantly reduce transmission.[19] The adoption of routine preventive behaviors based on appropriate training and control among health care workers (HCWs), undertaken prior to the isolation of SARS patients, was shown to be one of the most crucial control measures.[20–22]

In this context, Vietnam is considered to have achieved the first highly successful containment of SARS during the early phase of the outbreak.[23] One reason for this rapid containment is thought to be the prevention of infection leakage from hospitals back into the general community.[24] A second is the successful discontinuation of the chain of nosocomial transmission several days after onset based on the radical control measures of the Ministry of Health, Vietnam.[25] Although several nosocomial transmissions were observed in Hanoi French Hospital (HFH) in the early days of the outbreak,[26,27] none were identified in HFH or another local hospital in the latter phase.[28] In both hospitals, staff instituted stringent precautions, strict isolations, and quarantines under the encouragement of Dr. Carlo Urbani (Dr. Urbani died of SARS before seeing the success of the containment).[29] We therefore consider that a comprehensive understanding of the successful containment measures adopted by HFH and their theoretical underpinnings are crucial to the success of control strategies for any future recurrence. Here, we use a case-control study design to time-dependently examine the relationship between SARS and the precautionary behaviors undertaken by those exposed in HFH. We then use mathematical approaches to develop intuitive analyses of the impact of individual behaviors on the control of a SARS epidemic.

MATERIALS AND METHODS

Case-control study. HFH is a 56-bed secondary care hospital. After the admission of an index case on February 26, 2003, 38 cases in total were confirmed to have symptomatic SARS infection. The occurrence of newly diagnosed SARS cases due to local transmission continued until April 7, 2003, 3 weeks before the date when the Vietnamese government and WHO declared the outbreak successfully contained (April 28, 2003) (Table 1). The duration of the HFH outbreak was analyzed by separating it into three phases: Stage 1, February 26–March 4, from admission of the index case to the onset of secondary cases; Stage 2, March 5–March 10, from the suspicion of nosocomial spread to closure of the hospital; and Stage 3, from March 11 on, from strict isolation to local eradication.

A case-control study of 29 of the 38 laboratory-confirmed SARS cases and 98 controls was performed in HFH. The case group included 22 of 28 (78.6%) individuals admitted and retained in HFH and 7 of 10 (70.0%) individuals transferred to another hospital after first being admitted to HFH (total N = 29). The reasons for nonparticipation were death due to

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TABLE 1
Chronology of the outbreak of SARS in Hanoi French Hospital (HFH), Vietnam

Stage 1		
26-Feb-03	Day 0*	An index case complaining of fever, dry cough, and headaches was admitted to HFH.
2-Mar-03	Day 3	After intubation, the index case was isolated in ICU the following day.
4-Mar-03	Day 6	Nine secondary cases were suspected.
Stage 2		
5-Mar-03	Day 7	Seven additional cases were suspected. HFH informed the Ministry of Health, Vietnam, of the strange influenza. The health minister and experts from the World Health Organization (WHO) held a meeting. Dr. Carlo Urbani informed all staff to perform stringent precautions.
8-Mar-03	Day 10	HFH decided to close all outpatient/inpatient services. Visitors were not allowed to enter HFH. The hospital board of directors held an emergency meeting. Dr. Carlo Urbani explained the necessity of precautions and possibility of contamination as a mode of transmission. Health care workers were advised not to return home.
Stage 3		
11-Mar-03	Day 13	All inpatients were transferred to other hospitals. The 2nd floor of HFH was allocated to SARS patients only and strict isolation was enforced. • Three zones were allocated according to symptoms. • Nonmission individuals including health care workers were not allowed to enter.
13-Mar-03	Day 15	A special committee for SARS control and prevention was established. WHO issued a "global alert" to worldwide health authorities.
28-Apr-03	Day 60	The Vietnamese government and WHO declared successful containment of SARS in Vietnam.

* Day, days after onset of the outbreak. SARS, severe acute respiratory syndrome; ICU, intensive care unit.

SARS and/or respiratory failure (N = 5, 13.2%), refusal to take part (N = 1, 2.6%), or relocation (N = 3, 7.9%). The case group included 28 HFH employees (3 doctors, 13 nurses and nursing assistants, 10 radiologists and other co-medical workers, and 2 receptionist and administrative staff) and 1 relative of a patient. A further 23 Vietnamese patients who were directly admitted to another hospital were excluded because the detailed source of infection was unknown, although several cases were thought to have been infected in HFH. Detailed descriptions of the laboratory diagnoses were given previously.[28] They were confirmed through serological studies using an indirect enzyme-linked immunosorbent assay (ELISA) (Kirikae T, et al., unpublished data).

Controls were nominated based on employment in HFH and exposure among patients' relatives through HFH. The selection criteria included i) Vietnamese individuals more than 20 years old, ii) those who provided written informed consent based on explanation of our methods and purposes, and iii) those thought to have had contact with confirmed cases inside the hospital based on contact investigations. In total, 98 individuals were included as controls; most were HFH employees (13 doctors, 20 nurses and nursing assistants, 13 radiologists and other co-medical workers, and 11 receptionists and administrative staffs) or relatives of patients (N = 41). Although we investigated certain known contacts for inclusion as controls, namely individuals who took care of cases or entered cases' room, those who might have had trivial contact, such as possible exposure outside the hospital during, for example, transportation of SARS cases or in the casualty reception room, were not followed and included. The number of hospital employees investigated represented approximately 55.9% of the total employees used during the outbreak.

All participants were surveyed with regards to their use of personal protective equipment (PPE) and hygiene habits when in contact with patients with SARS; that is, the use of masks, gloves, and gowns, and the practice of hand washing, which were specifically recommended as droplet and contact precautions. In this paper, masks denote surgical masks; N95 masks were not available in the early stage of the outbreak in Vietnam. Individual behaviors were investigated mainly in two separate phases according to time-dependency (in Stage 1 and after entering Stage 2; i.e., Stages 2 and 3) (Table 1) to clarify any behavioral changes that occurred. Standardized questionnaires requiring one of two possible answers for each precaution ("performed" or "not performed") were given to each subject, and all responses were collected. Answers of "sometimes" or "seldom" were defined as "not performed" due to imperfect efficacy. In addition, the frequency of contact with infected individuals was investigated to represent the number of exposures per day. An exposure result of "many times" was recorded for those who had close contact with SARS patients, that is, those who cared for or lived with SARS patients, and those likely to have come into direct contact with the respiratory secretions or body fluids of SARS patients, for example, during close conversation (within 3 feet).[30] After completing the initial primary survey, an identical confirmation survey was performed to confirm the validity of the answers. These surveys were conducted along with other epidemiologic studies (Nishiyama A, et al., unpublished data) until mid-March 2004, almost 1 year after onset of the epidemic. No blood test results showing possible asymptomatic infections were available during the survey period. The participants were informed of how the information would be used and assured of the confidentiality of their responses. The purpose of the study was explained in Vietnamese, and written informed consent was obtained.

Statistical analyses were performed as follows. First, univariate associations between precautionary behaviors and infection were investigated in two separate stages (Stage 1 or Stages 2 and 3). Comparisons between groups were made using the χ^2 or Fisher's exact test for univariate analysis. Multivariate logistic regression was done in Stage 1 using forward stepwise selection (Waldesian) to determine the most significant variable associated with protection among those studied. Significant steps were taken to minimize recall bias with Stages 2 and 3 data. Analysis was restricted to those who had probable contact in these stages. It was further restricted to those cases developing symptoms whose incubation period

was within the greater than 95% confidence interval (95% CI) of having occurred after the beginning of Stage 2; and finally to medical doctors and nurses only, for both cases and controls. Second, univariate associations between sociodemographic variables (sex, age, and occupation) and SARS were investigated, with age and occupation categorized into four different groups each. Third, interactions between the identified most significant protective behavior and other variables significantly associated in univariate analysis were investigated through the use of crosstabs statistics, in which the odds of being infected were stratified according to a comparison of variables, and interactions were sought through the different odds ratio in each strata. Finally, multiple logistic regression analysis was used to determine the protective effect and eliminate confounding variables. As described in the next section, all variables significantly associated in univariate analyses, as well as sociodemographic variables, were selected and entered together in the final model. All data were entered into Microsoft Excel 2000 (Microsoft Co., Redmond, WA), and the statistical data were analyzed using the statistical software "R" (R Development Core Team, Vienna).[31]

Mathematical methods. The predictive effects of the behavioral changes were simulated using an individual-based stochastic model. For ease of understanding, a compartmental model, a type of SEIR (susceptible [S], exposed [E], infected [I], and recovered/removed [R]) model, which considered the process of transmission according to the protective behaviors taken against infectious contact among susceptible individuals, was applied. Instead of assuming "exposed (latent)" and "infectious" periods, E and I were defined as "incubation" and "symptomatic" periods, respectively, as the infectious period of SARS has not been fully clarified. Although SEIR models are usually deterministic and use mean estimations as model parameters, even with regard to SARS,[12,32] stochastic simulations were performed in this study because of the need to consider the stochasticity of each protective behavior, and also because of the small sample population size. The infectious lifetime of each individual was presented as an absorbing Markov chain. The simulations start with an individual index case (Day 0) in a population of 300 in which all individuals are susceptible.

Of the total 127 subjects studied (29 cases and 98 controls), 62.2% (N = 79) were considered to have had casual contact and 37.8% (N = 48) to have had close contact with SARS patients. The number of casual contacts (κ_1) was directly obtained ($= 0.7 \pm 0.2$ [day^{-1}]), while the mean of close contacts ($\kappa_2 = 0.4$ [day^{-1}]) was determined with the following equation:

$$\kappa_2 = \kappa_1 \ln(\mathrm{OR}_{\mathrm{closed}}) \quad (1)$$

where $\mathrm{OR}_{\mathrm{closed}}$ (= 2.5; 95% CI: 1.1–5.9) denotes the odds ratio (OR) of getting infected as a result of close contact. In other words, to quantify close contact, we assumed that the frequency of infection is mainly determined by the frequency of contact, so that the ratio of the frequency of close to casual contact becomes proportional to the logarithm of the OR of transmission. The protective effect of precautionary behavior was approximated by:

$$\beta = 1 - \mathrm{RR} = 1 - \frac{a(c+d)}{c(a+b)} \approx 1 - \frac{ad}{bc} \approx 1 - \mathrm{OR} \quad (2)$$

where RR and OR denote the relative risk and odds ratio, respectively, of becoming infected while performing a protective behavior (with precaution = with exposure). Here, a is the number of exposed ill people; b, the number of exposed healthy people; c, the number of unexposed ill people; and d, the number of unexposed healthy people. If the outcome (i.e., disease investigated) is a rare event, that is, if a and c are very small compared with b and d, respectively, $(a + b)$ and $(c + d)$, respectively, would be closely similar to b and d alone. In this case, OR would approximate RR.

The lengths of the incubation and symptomatic periods were both assumed to be independently and identically distributed random variables with a probability density function of γ distribution, the mean and variance of which were defined as 3.8 [days] and 8.3 [days^2], and 16.2 [days] and 7.9 [days^2], respectively.[24,33] These distributions were applied to difference equations (as a discrete time model) by discretizing the probability density functions by day (for a detailed description of the simulation algorithm, see the Appendix).

The first simulation scenario hypothetically investigated the unchanged coverage and mean protective effects of a behavioral measure throughout the epidemic. Primary information on protective behaviors was obtained from our Stage 1 survey. Estimates for the extent of a protective effect, the associated causative behavior of which was found in forward stepwise logistic regression to be the most significantly associated with protection (as described above), were obtained through the use of further multivariate logistic regression analysis. This analysis incorporated all variables significantly associated with SARS on univariate analysis (i.e., other precautionary behavior, gender, age and occupation). To investigate the impact of the coverage of a protective measure on the trajectory of an outbreak, sensitivity of the cumulative number of SARS cases at Day 30 to the coverage of masks was investigated in the mean field equation. In the second scenario, it was assumed that coverage improved dramatically after entering Stage 2 (Day 7) due to an awareness of transmission. Further, in Stage 3 (Day 13), the hospital implemented not only stringent precautions but also strict isolations. To understand the trajectory of transmission in detail, the number of incubating as well as symptomatic individuals was investigated. As was in fact seen during Stage 3 of the outbreak, it was also assumed that all cases who became symptomatic were immediately isolated and that nobody except a limited number of healthcare workers were permitted to have contact with them. Because the greatest uncertainty applies to the time taken to increase coverage of a protective measure and to implement strict isolations, sensitivity analyses comparing the cumulative number of SARS cases up to Day 30 were performed with the time to change both protective measures set simultaneously on the same day. Finally, the basic reproduction number was estimated using the (effective) reproduction number obtained in Stage 1 (see Appendix).

RESULTS

Table 2 shows the univariate association between the precautionary behaviors taken (SARS and non-SARS [control] cases) in Stage 1 and SARS. The use of masks (P = 0.011) and gowns (P = 0.012) appeared to prevent infection, whereas handwashing and the use of gloves were less likely to provide protection. Only two subjects who performed all pro-

TABLE 2
Precautionary measures taken by all participants in Stage 1

	SARS cases (N = 25)	Non-SARS (N = 90)	P value*	Odds ratio† (95% CI)‡
All measures	2	44	0.059	0.2 (0.0–1.0)
Handwashing before§	12	51	0.937	1.0 (0.4–2.3)
Handwashing after¶	15	56	0.766	1.1 (0.5–2.8)
Masks‖	8	35	0.011	0.3 (0.1–0.7)
Gloves	8	30	0.643	0.7 (0.3–1.9)
Gowns	2	25	0.012	0.2 (0.0–0.8)

* Two-tailed.
† Odds ratio of being infected while taking specific precautions.
‡ 95% CI: 95% confidence interval.
§ Hands washed before having contact with a patient.
¶ Hands washed after having contact with a patient.
‖ Only those who always used a mask.

tective measures developed symptomatic infections (P = 0.059). Forward stepwise logistic regression of the five protective measures (0.05 for entry and 0.10 for removal probability) showed that only the use of masks was significant in the final model (OR, 0.29, 95% CI; 0.11-0.73, P = 0.009). In Stages 2 and 3, the use of masks (P = 0.001) and gowns (P = 0.010) was significantly associated with non-infection among doctors and nurses still not infected after Stage 1 (Table 3). Most performed all the personal protective measures recommended, and only one individual who wore masks was infected. The comparative results of the behaviors of all participants at Stage 1 and after entering Stage 2 are shown in Figure 1a. The proportions of individuals who performed the investigated protective behaviors increased after entering Stage 2. However, these behavioral changes were not significantly different between the two phases (P = 0.960). The behaviors performed by the doctors and nurses (N = 48; Figure 1b) who had the closest contact with the SARS patients drastically and significantly improved after entering Stage 2 (χ^2 = 9.855, P = 0.043).

The univariate associations between socio-demographic variables and SARS throughout the epidemic are shown in Table 4. Females were more likely to become infected than males (P = 0.011), and a significant association of SARS with nurses (P = 0.008) was observed. In HFH, infection was frequent in the 40–49 age strata (P = 0.015). Among all study subject, relatives of patients (P < 0.001) appeared to be the least frequently infected. Table 5 shows the interaction between the use of masks and other significantly associated variables in univariate analyses. Even though we saw no significant difference in the OR of using masks versus the use of gowns, females (OR = 0.2) and nurses (OR = 0.1) were more effectively protected by the use of masks than others in Stage 1. In Stages 2 and 3, the use of gowns showed overall reasonable OR (= 0.2), whereas most other interactions could not be calculated due to the scarcity of cases.

TABLE 3
Precautionary measures taken by health care workers in Stages 2 and 3

	SARS cases (N = 4)	Non-SARS (N = 26)	P value*	Odds ratio† (95% CI)‡
All measures	1	25	0.001	< 0.1 (0.0–0.3)
Handwashing before§	4	25	1.000	NC
Handwashing after¶	4	25	1.000	NC
Masks‖	1	25	0.001	< 0.1 (0.0–0.3)
Gloves	4	25	1.000	NC
Gowns	3	26	0.010	NC

* Two-tailed.
† Odds ratio of being infected while taking specific precautions.
‡ 95% CI: 95% confidence interval.
§ Hands washed before having contact with a patient.
¶ Hands washed after having contact with a patient.
‖ Only those who always used a mask.

Figure 2a shows the mean and corresponding 95% CI of the trajectory (shown as prevalence) of an epidemic from 250 simulation runs which hypothetically assumed unchanged coverage as well as the protective effects of the precautionary measures observed in Stage 1. The precautionary measure in this simulation was based on a multivariate logistic regression which included all variables showing significant associations in univariate analyses, and focused on the impact of the use of masks, given the identification of this behavior as the most important protective measure (β = 0.6 obtained from OR = 0.4, P = 0.020). The coverage of masks was obtained as 52.0% from Table 2. If an outbreak was simply allowed to continue growing under these conditions, the results showed that approximately 50 to 90 symptomatic cases would occur by Day 30. The reproduction number (R) was estimated as 4.1 (95% CI; 1.9–6.4), and from this estimate the basic reproduction number was estimated as 6.0. Sensitivity of the cumulative number of cases to the coverage of masks, in the mean field, is shown in Figure 2b. Certain reduction in the cumulative number of cases was observed with significant improvements in coverage.

Figures 2c and 2d shows the outbreak trajectory of 250 simulations assuming improved coverage (from 52.0 to 81.5%) among susceptible individuals on Day 8 and restriction of contact with symptomatic individuals to health care workers on Day 13. The protective effect obtained from multivariate regression was 0.9 (OR = 0.1, P = 0.955). The reproduction number in Stage 2 was estimated as 0.7 (95% CI; 0.0–2.3). The number of incubating individuals began to show a decreasing trend after these events (Figure 2c), followed by a declining trend in the number of symptomatic cases (Figure 2d). Most of the simulated outbreaks eventually declined to extinction before Day 120. The sensitivity of the final size of an epidemic, evaluated through observations of the cumulative numbers of cases, to the timing of drastic changes in protective behaviors accompanied by strict isolation is shown in Figure 2e. When the stochastic effects are taken into account together with the effects of single precautionary measures and isolation, the rapid implementation of combined measures reduces the number of transmissions and increases the probability of extinction.

DISCUSSION

The findings of this case-control study indicate that the use of masks was significantly associated with the prevention of SARS transmission and that precautions against droplet contamination and contact were adequate in preventing transmission; this implies mainly to in-hospitals. The results are roughly consistent with those of previous reports.[18,20,22] Although a number of exceptions were seen with regard to protective effects during patient intubation, during which transmission to staff occurred even when droplet and contact precautions were taken,[7,34] one of the most important lessons from the SARS outbreak is the need to enhance infection control programs in hospitals.[13,35] Even though the use of

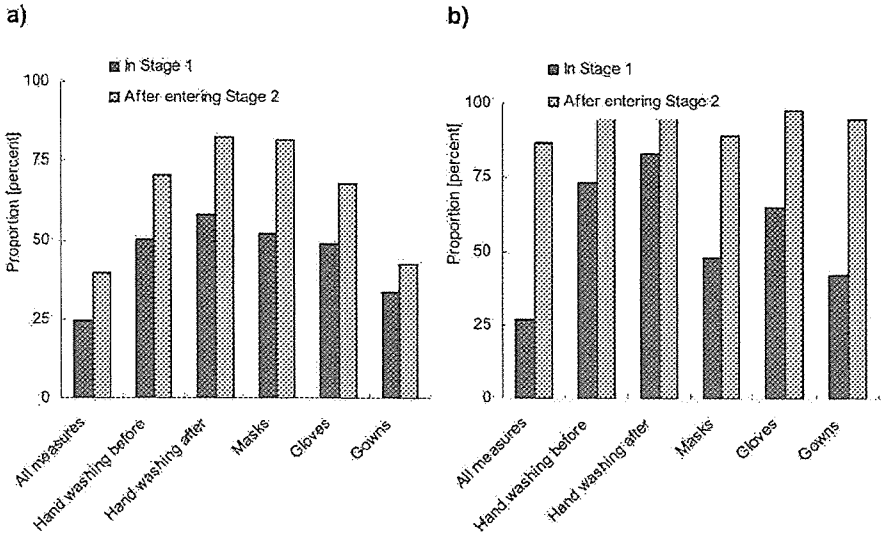

FIGURE 1. Protective behavioral changes defined by stage. **a**, Proportion of participants (SARS and non-SARS [control] cases) who performed each precautionary measure in Stage 1 (N = 127) and after entering Stage 2 (N = 108). Handwashing "before" and "after" denote before and after contact with a patient, respectively. **b**, Proportion of health care workers who performed each precautionary measure in Stage 1 (N = 48) and after entering Stage 2 (N = 37).

masks was the most effective precautionary measure, masks alone together with the observed coverage did not reduce the reproduction number below unity (R_0 = 6.0 and R with the protective effects of masks = 4.1). Put simply, the use of masks alone was shown to be insufficient to contain the epidemic. Further, it was shown that the coverage of precautionary behaviors among the study subjects increased with the progression of the outbreak, and this was especially obvious among doctors and nurses. In HFH, remarkable changes occurred in the very early phase of the outbreak before detailed information about SARS was available. According to the stochastic simulations, an increased probability of extinction would be observed if the combined measures of precaution and isolation were rapidly implemented.

With regard to sociodemographic variables, females were more frequently infected than males. Given that transmission was most frequently observed among nurses, a plausible explanation for this finding would be occupational background. Although the 40–49 age group was frequently infected, we have no persuasive explanation for this apart from occupation: 61.9% of this stratum was medical doctors or nurses. Considering that nurses were more effectively protected from transmission by the use of masks, the control measures taken by them within HFH from early in the epidemic were admirable. The lowest frequency of infection was seen in relatives of patients, showing that our study included many relatives who remained uninfected but were nevertheless believed to have had contact. Because nonmatched case-control designs such as this are vulnerable to selection bias, we obtained estimates of the protective effect of masks by means of multivariate logistic regression analysis which entered all other variables significantly associated with infection in univariate analysis. After adjustment for internal confounding variables, the estimated reproduction number was given as 0.7 in Stages 2 and 3. Previous studies have shown that the (effective) reproduction number, defined as the average number of secondary cases generated by one index case in a susceptible population under certain restrictions and interventions, decreases with increasing awareness of the epidemic combined with several public health measures.[36,37] Using reasonable estimation procedures, another study showed that R significantly decreased after a global alert in most affected countries.[38] The current study showed that the estimated R decreased below unity after notification of a hospital outbreak, although the estimates were obtained using rough assumptions and the process of estimation was biased by various factors.

In HFH, the rapid increase in awareness, which led to not only strengthened precautionary measures and isolation but also quarantining of health care workers, seems to have been the greatest contributor to successful containment. One reason for this quick response could be attributed to the background of secondary cases that arose mainly from health care workers who had close contact with the index case. Almost all staff members working or on duty in the earliest days of the

TABLE 4

Univariate associations between age-class/occupational categories and SARS

	Category	N	P value*	Odds ratio (95% CI)†
Sex	Male	47	0.011	0.3 (0.1–0.8)
	Female	70	0.011	3.3 (1.2–9.0)
Age class	29 y/o	29	1.000	0.9 (0.3–2.3)
	30–39 y/o	44	0.080	0.4 (0.2–1.1)
	40–49 y/o	42	0.015	2.8 (1.2–6.6)
	50 y/o	12	0.733	0.7 (0.1–3.2)
Occupation	Medical doctors	16	1.000	0.8 (0.2–2.9)
	Nurses	33	0.008	3.2 (1.3–7.7)
	Other co-medicals	36	0.076	2.2 (0.9–5.2)
	Relatives of patients	42	< 0.001	< 0.1 (0.0–0.4)

* Two-tailed.
† Odds ratio of being infected while taking specific precautions.

TABLE 5
Interactions between wearing masks and other variables on the infection

	In stage 1			In stages 2 and 3		
	Odds for masks (+)	Odds for masks (−)	Odds ratio*	Odds for masks (+)	Odds for masks (−)	Odds ratio*
Gowns						
(+)	0.3	0.6	0.5	< 0.1	2.0	0.2
(−)	0.3	0.5	0.6	NC	NC	NC
Sex						
(male)	0.1	0.2	1.0	0.0	0.0	NC
(female)	0.2	0.8	0.2	0.1	NC	NC
Age class						
29 y/o	0.1	0.4	0.3	0.0	NC	NC
30–39 y/o	0.1	0.3	0.5	0.0	NC	NC
40–49 y/o	0.3	0.8	0.3	0.1	1.0	0.1
50 y/o	0.2	0.2	1.0	0.0	NC	NC
Occupation						
(Medical doctors)	NC	0.6	NC	0.0	0.0	NC
(Nurses)	0.2	1.6	0.1	0.1	0.0	NC
(Other co-medicals)	0.5	0.5	1.2			
(Relatives of patients)	NC	0.1	NC			

NC = not calculable.
* Odds ratio of being infected while taking specific precautions.

outbreak (in Stage 1) were severely infected.[39,40] Another reason might be due to the efforts led mainly by Dr. Carlo Urbani, who suggested quick improvements in the precautionary measures taken and isolation.[29] As a result, transmission leakage into the community was prevented, thus having a huge impact on the chains of transmission.[24] In HFH, those who were exposed implemented precautionary and other controlling measures quickly and efficiently, and the epidemic consequently declined to extinction.

In the interests of objective interpretation, the limitations of our study design must be addressed, as follows:

1) A study such as ours in which exposure has a strong intuitive causal link with outcome (i.e., mask usage) is vulnerable to recall bias. Even though we limited our subjects in Stages 2 and 3 to medical doctors and nurses, and cases were appropriately selected according to the probable date of infection and incubation period, our estimates are likely less accurate than would be obtained by blinded or matched case-control study. In addition to this directional bias, further bias may have been introduced by random misclassification, as our records were completed 1 year after the outbreak, and it is therefore possible that some of the precautions were uncertain exposures. The frequent use of masks among controls may have reduced the strength of the associations.
2) Model-generated results must be interpreted cautiously. Although the simulations shown here included only the effect of masks and were considered according to the results of multivariate logistic regression adjusted for internal factors, unknown external confounding factors likely exist. For example, in Stages 2 and 3, although multivariate logistic regression was performed with other variables, the P value obtained was 0.955, and overall the model was weak. Owing to the scarcity of case records, stratification in this stage failed to separate the effects of masks. Thus, the estimates of the protective effect of masks and reproduction number in this stage may include the effects of other concomitant changes, such as the reduced frequency of contacts and quarantine.
3) There are limitations concerning the simplicity of our model; for example, we neglected the possible differential susceptibility of humans to asymptomatic infections,[41,42] individual variance in severity and/or prognosis,[23,43,44] and the highly heterogenous transmission of SARS.[4,5,45] Theoretical exercises never replace reality.
4) Finally, because our model was based on a case-control study, the estimates of coverage were biased; principally, coverage in a case-control design is taken from a nonrepresentative sample. Although this study was conducted as a first attempt to incorporate the effect of behavioral factors, which change time-dependently, to model building strategies for the control of directly transmitted airborne diseases, further studies incorporating a number of methodological improvements are required.

In conclusion, given that early recognition that leads to the implementation of protective behaviors and effective control strategies is crucial in hospitals,[46] we believe our model provides intuitive results that at least partly satisfy the need to evaluate outbreak trajectories based on individual behaviors.

APPENDIX

Each simulation starts with one index case and is based on a model constructed as follows:

i) The expected number of people who used protection on each subsequent day was determined by the number of susceptible individuals (S), number of contacts per day (κ), proportion of individuals who performed the protective behavior (p), and the protective effect of the precautionary measure (β), which were obtained based on our survey. The number of infectious contacts, denoted by the product of the number of susceptible individuals (S) and the mean number of contacts (κ), was divided into two subgroups: one that represents protection due to precautionary behaviors against infection with SARS-CoV (SARS-associated coronavirus) and another that does

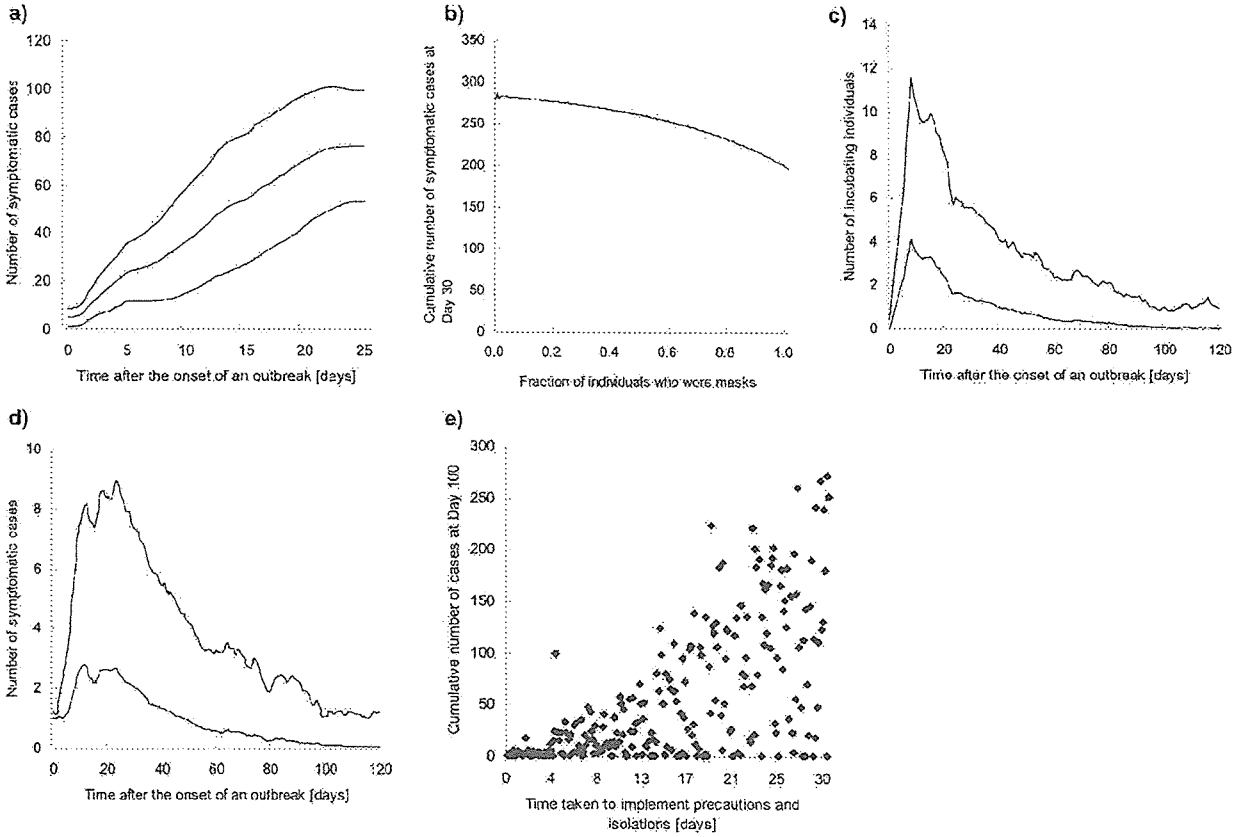

FIGURE 2. Stochastic simulations of a SARS outbreak with dependency on the coverage and protective effect of precautionary behaviors. **a**, Predicted number of symptomatic cases and corresponding 95% confidence interval (95% CI) given by 250 simulation runs assuming unchanged (stable) protective behaviors for the entire period. The reproduction number (R) was 4.1 ± 1.1. **b**, Sensitivity of the cumulative number of cases at Day 30 to the coverage of masks. The obtained line represents the simulation based on mean field (without assuming random function with binomial distribution in each transition probability). The protective effect of wearing a mask was fixed ($\beta = 0.6$). **c** and **d**, Stochastic simulations of a SARS outbreak with dependency on a combination of precautionary measures and strict isolation. **c**, The mean number of incubating individuals and corresponding 95% CI from 250 runs with changes in protective behaviors combined with strict isolation (lower 95% CI is x-axis). At Day 7, the effectiveness/coverage of precautionary measures used improved from 0.6/52.0 to 0.9/89.2, respectively. At Day 13, the number of susceptible individuals decreased from 300 to 20. The reproduction number decreased from 4.1–0.7 ± 1.1–0.8. **d**, The mean ± 95% CI of symptomatic cases given by 250 runs assuming changes in protective behaviors combined with strict isolation. The conditions were the same as those in **c**. **e**, Sensitivity of the size of an outbreak (represented by the cumulative number of cases) to the time taken to enhance precautionary measures and implement strict isolation; the combined measures are started at the same time and under the same conditions as in **c**.

not, according to $(1 - p\beta)$. However, these groups were not permanently fixed. The mean of the number of contacts based on our survey was approximated by:

$$\kappa = \kappa_1\pi_1 + \kappa_2\pi_2 = \kappa_1\pi_1 + \kappa_1 \ln(\mathrm{OR}_{close})\pi_2 \qquad \text{(A1)}$$

where κ_1, κ_2, π_1, and π_2 denote the number of casual and close contacts and the fraction of individuals who had casual and close contacts, respectively, while the odds ratio of getting infected with close contact is represented by OR_{close} and N, respectively.

ii) Both the incubation (E) and symptomatic (I) periods were assumed to be independently and identically distributed following an approximated probability density function with gamma distributions[33] (denoted by γ_k and c_l for the discretized stages [days] k and l, respectively). We divided the probability density functions into k ($i = 14$) and l ($j = 12$) stages; the methodology of approximation by date was previously reported.[23] The relative measure of infectiousness for the incubation (E) period (q) was assumed to be 0.1.[12]

iii) Based on realistic settings in Vietnam, it was assumed that all individuals were isolated with the onset of early signs of clinical symptoms under the isolation measures; and for simplicity, the effect of quarantine was neglected. When considering strict isolation, the number of susceptible individuals having contact with SARS patients was limited to 20 (which is the approximate number of ward workers); the number of susceptible individuals was treated as being stable (always $S = 20$) so that S would not be exhausted thereafter; without isolation there were assumed to be 300 susceptible individuals (which is roughly the total number of people involved in possible contacts in HFH). $N = S + E + I + R$, and background mortality was neglected. The resulting simplest difference equations were formulated as follows:

$$
\begin{aligned}
S(t+1) &= \exp\left[-\kappa(1-p\beta)\frac{I+qE}{N}\right]S(t)\\
E_1(t+1) &= \left\{1-\exp\left[-\kappa(1-p\beta)\frac{I+qE}{N}\right]\right\}S(t)\\
E_k(t+1) &= (1-\gamma_{k-1})E_{k-1}(t)\\
I_1(t+1) &= \sum_{k=1}^{i}\gamma_k E_k(t)\\
I_l(t+1) &= (1-c_{l-1})I_{l-1}(t)\\
R(t+1) &= R(t)+\sum_{l=1}^{j}c_l I_l(t)
\end{aligned}
\qquad \text{(A2)}
$$

Based on the forward stepwise logistic regression result in the case-control study, and to facilitate understanding, p and β were used only to represent the use of masks. However, the protective effect, β, was obtained from the result of further multiple logistic regression which entered all other significantly associated variables (in univariate analysis). All terms shown here as products of a probability and a state variable were generated in our simulations by using random variables with binomial distributions. Under these assumptions and using mean length of incubation and symptomatic periods, the reproduction number (R) is given by:

$$R = \kappa(1-p\beta)\left(\frac{q}{\gamma}+\frac{1}{c}\right) \qquad \text{(A3)}$$

where γ^{-1} and c^{-1} are the means of the incubation and symptomatic periods in days, respectively. The basic reproduction number was estimated by

$$R_0 = \frac{R}{(1-p\beta)} \qquad \text{(A4)}$$

For the purpose of mathematical convenience, although unrealistic, our model assumed homogenous mixing as well as all infectious individuals being equally infectious.

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Identification of an Alternative 5′-Untranslated Exon and New Polymorphisms of Angiotensin-Converting Enzyme 2 Gene: Lack of Association With SARS in the Vietnamese Population

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We analyzed genetic variations of angiotensin-converting enzyme 2 (ACE2), considering that it might influence patients' susceptibility to severe acute respiratory syndrome-associated coronavirus (SARS-CoV) or development of SARS as a functional receptor. By cloning of the full-length cDNA of the *ACE2* gene in the lung, where replication occurs on SARS-CoV, it was shown that there are different splicing sites. All exons including the new alternative exon, exon-intron boundaries, and the corresponding 5′-flanking region of the gene were investigated and 19 single nucleotide polymorphisms (SNPs) were found. Out of these, 13 SNPs including one non-synonymous substitution and three 3′-UTR polymorphisms were newly identified. A case control study involving 44 SARS cases, 16 anti-SARS-CoV antibody-positive contacts, 87 antibody-negative contacts, and 50 non-contacts in Vietnam, failed to obtain any evidence that the *ACE2* gene polymorphisms are involved in the disease process in the population. Nevertheless, identification of new 5′-untranslated exon and new SNPs is considered helpful in investigating regulation of ACE2 gene expression in the future.

KEY WORDS: angiotensin-converting enzyme 2 (ACE2); severe acute respiratory syndrome (SARS); SARS associated coronavirus (SARS Co-V); virus receptor; polymorphism; association study

INTRODUCTION

Severe acute respiratory syndrome (SARS) is an emerging infectious disease characterized by systemic inflammation followed by atypical pneumonia [Peiris et al., 2003b]. Shortly after the initial worldwide outbreak in 2003, SARS-associated coronavirus (SARS-CoV) was discovered as an etiological agent of SARS [Drosten et al., 2003; Ksiazek et al., 2003; Kuiken et al., 2003; Peiris et al., 2003a], and then angiotensin-converting enzyme 2 (ACE2) was identified as a functional receptor of this newly arrived virus [Li et al., 2003]. More recently, CD209L was reported as being another alternative receptor for the virus, but it appears to be a less efficient entry site than ACE2 [Jeffers et al., 2004].

Virus receptors generally play a key role in the entry of the pathogen into the host cells and may influence development or progression of viral diseases. For example, it is well known that genetic polymorphism of chemokine receptor 5 (CCR5), a co-receptor for human immunodeficiency virus-1 (HIV-1), influences the natural history of HIV-1 infection. The mutant allele CCR5-Δ32 does not produce a functional protein and has been shown to protect host cells against HIV-1 infection, and progression into acquired immunodeficiency syndrome is delayed after seroconversion takes place [Dean et al., 1996; Liu et al., 1996; Samson et al., 1996]. By analogy with the above, we considered that genetic polymorphisms of ACE2 could influence SARS-CoV infection or clinical manifestations of SARS.

ACE2 is a homologue of ACE1 and exhibits 40% identity of amino acid sequence to its N- and C-terminal domains [Tipnis et al., 2000]. Similar to ACE1, ACE2 is a metalloprotease that constitutes a renin-angiotensin system. Human full-length ACE2 cDNAs have been cloned already from lymphoma (GenBank accession No. AF241254) [Tipnis et al., 2000], cardiac left ventricle (AF291820) [Donoghue et al., 2000] and testis (AY623811) [Douglas et al., 2004]. Based on published data, it has been said that the ACE2 gene (*ACE2*) contains 18 exons, and spans approximately 40 kb of genomic DNA on the human X-chromosome. Although *ACE2* mRNA expressions were demonstrated in the lung by the method of quantitative reverse transcription-PCR (RT/PCR) [Harmer et al., 2002] and its protein expression was obviously shown by immunohistochemistry [Hamming et al., 2004], full-length *ACE2* cDNA has not been cloned from the lung so far. This is considered to be

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