MAJOR ARTICLE

Association of *ICAM3* Genetic Variant with Severe Acute Respiratory Syndrome

Kelvin Y. K. Chan,[1] Johannes C. Y. Ching,[1] M. S. Xu,[1] Annie N. Y. Cheung,[1] Shea-Ping Yip,[9] Loretta Y. C. Yam,[10] Sik-To Lai,[11] Chung-Ming Chu,[12] Andrew T. Y. Wong,[11] You-Qiang Song,[2,7] Fang-Ping Huang,[1] Wei Liu,[1] P. H. Chung,[13] G. M. Leung,[8] Eudora Y. D. Chow,[12] Eric Y. T. Chan,[1] Jane C. K. Chan,[14] Hextan Y. S. Ngan,[3] Paul Tam,[4,7] Li-Chong Chan,[1] Pak Sham,[5] Vera S. F. Chan,[15] Malik Peiris,[6] Steve C. L. Lin,[15] and Ui-Soon Khoo[1]

Departments of [1]Pathology, [2]Biochemistry, [3]Obstetrics and Gynecology, [4]Surgery, [5]Psychiatry, and [6]Microbiology, and [7]Genome Research Center, Hong Kong Jockey Club Clinical Research Centre, and [8]Department of Community Medicine, Li Ka Shing Faculty of Medicine, University of Hong Kong, [9]Department of Health Technology and Informatics, Hong Kong Polytechnic University, [10]Pamela Youde Nethersole Hospital, [11]Princess Margaret Hospital, [12]United Christian Hospital, [13]Hong Kong SAR Department of Health, and [14]Hospital Authority Severe Acute Respiratory Syndrome Collaborative Group, Hong Kong Hospital Authority Head Office, Hong Kong; [15]Department of Biosurgery and Surgical Technology, Imperial College London, London, United Kingdom

Genetic polymorphisms have been demonstrated to be associated with vulnerability to human infection. *ICAM3,* an intercellular adhesion molecule important for T cell activation, and *FCER2 (CD23),* an immune response gene, both located on chromosome 19p13.3, were investigated for host genetic susceptibility and association with clinical outcome. A case-control study based on 817 patients with confirmed severe acute respiratory syndrome (SARS), 307 health care worker control subjects, 290 outpatient control subjects, and 309 household control subjects unaffected by SARS from Hong Kong was conducted to test for genetic association. No significant association to susceptibility to SARS infection caused by the novel coronavirus (SARS-CoV) was found for the *FCER2* and the *ICAM3* single nucleotide polymorphisms. However, patients with SARS homozygous for *ICAM3 Gly143* showed significant association with higher lactate dehydrogenase levels ($P = .0067$; odds ratio [OR], 4.31 [95% confidence interval {CI}, 1.37–13.56]) and lower total white blood cell counts ($P = .022$; OR, 0.30 [95% CI, 0.10–0.89]) on admission. These findings support the role of *ICAM3* in the immunopathogenesis of SARS.

Severe acute respiratory syndrome (SARS) caused by the novel coronavirus (SARS-CoV) occurred largely in Chinese communities and in Asian countries [1]. A proportion of persons exposed to the virus without adequate protection, however, did not develop the disease [2]. It has been reported that of the close contacts of all 1755 patients with SARS diagnosed and treated in Hong Kong, only 14% actually died of the disease; the reason for this has yet to be discovered. Genetic polymorphisms have been demonstrated to be associated with vulnerability to a variety of human infections, including SARS [3]. Association between susceptibility to SARS and the major histocompatibility complex (MHC) class I has also been reported (e.g., the *HLA-B*4601* allele [4] and *HLA-B*0703* allele from Hong Kong [5]). However, the subject numbers in both these studies had been small.

The angiotensin-converting enzyme–2 (ACE2) is the only known functional receptor for SARS-CoV infection/replication [6], but no significant association for susceptibility or clinical outcome has been shown with the polymorphisms of the *ACE2* gene [7]. DC-SIGN (dendritic cell [DC]–specific intercellular adhesion molecule-3 [ICAM3]–grabbing nonintegrin, encoded

Received 1 June 2005; accepted 16 February 2007; electronically published 5 June 2007.

Potential conflicts of interest: none reported.

Presented in part: Human Genome Meeting, 18–21 April 2005, Kyoto, Japan (abstract 17).

Financial support: Research Fund for the Control of Infectious Diseases, Hong Kong (project 03040792); Canadian Genetic Diseases Network Research Grant; National Institute of Allergy and Infectious Diseases (Public Health Research Grant AI 95357).

Reprints or correspondence: Dr. Ui-Soon Khoo, Rm. 324, 3/F, University Pathology Bldg., Dept. of Pathology, University of Hong Kong, Queen Mary Hospital, Pokfulam Rd., Hong Kong (uskhoo@pathology.hku.hk).

The Journal of Infectious Diseases 2007;196:271–80

0022-1899/2007/19602-0015$15.00
DOI: 10.1086/518892

Table 1. Demographic features of patients with severe acute respiratory syndrome (SARS) and control subjects.

Characteristic	Patients with SARS		Control subjects		
	Initially recruited (n = 309)	All (n = 817)	Health care workers (n = 307)	Household contacts (n = 309)	Outpatients (n = 260)
Age, years					
Mean (SD)	41.2 (14.3)	40.26 (13.8)	34.7 (9.6)	41.8 (14.3)	49.9 (19.8)
Median	40.0	38.0	33.0	43.0	47.0
Range	5–85	5–88	21–60	18–80	4–95
Sex					
Male:female	2:3	2:3	2:6.6	2:2.4	2:4
Female, no. (%)	188 (61.1)	505 (61.8)	235 (76.5)	168 (54.4)	173 (66.5)

by *CD209*) is an important C-type lectin expressed on DCs that can bind pseudovirus transfected by the SARS spike gene [8, 9]. L-SIGN (liver/lymph node–specific ICAM3 grabbing nonintegrin, encoded by *CLEC4M*) is a homologue of DC-SIGN and is also a binding receptor for SARS-CoV infection [10]. Interestingly, *DC-SIGN*, *L-SIGN*, and *FCER2 ICAM3* genes are all mapped on 19p13.3, within 71 kb of each other, and belong to the C-type lectin family [11], whereas ICAM3 is the natural ligand for both DC-SIGN and L-SIGN [12, 13].

ICAM3, being expressed constitutively on T cells and other leukocytes, is a potent signalling molecule and a major ligand in the initiation of T cell–mediated immune responses [14, 15]. In addition to the recognition of peptide/MHCs on the surface of professional antigen presenting cells (APCs), such as DC [16], by the T cell receptors on the T cell surface, the interaction of adhesion molecules between the APCs and T cells are also critical for activating antigen-specific T cells [17]. The binding between ICAM3 and its ligands DC-SIGN or leukocyte function–associated antigen–1 [18] provides transient engagement of naive T cells with DCs, which then allows the T cells to sample large numbers of MHC molecules for the presence of specific peptides [19]. This initial cell-to-cell engagement step is critical for induction of T cell responses, which in turn play a central role in the immunoregulation of infectious diseases. It is noteworthy that circulating forms of ICAM3 have been used as a parameter to monitor disease progression, particularly in HIV-infected patients [20]. However, the role of ICAM3 in settings of other viral infection, such as SARS-CoV infection, is still unknown.

As the association between specific MHC alleles and susceptibility to SARS has been observed, we hypothesized that polymorphisms of other related molecules such as *ICAM3, FCER2, DC-SIGN,* and *L-SIGN* may influence susceptibility to SARS-CoV infection. We have previously shown that individuals homozygous for the tandem neck repeats of *L-SIGN (CLEC4M)* gene are less susceptible to SARS infection [21]. In the present case-control genetic association study, we report that the *Gly143* polymorphism of *ICAM3* are associated with higher lactate dehydrogenase (LDH) levels and lower total white blood cell (WBC) counts on admission in patients with SARS-CoV infection.

PATIENTS, MATERIALS, AND METHODS

Subjects were recruited for this study after approval from the respective institutional review boards of hospitals involved. Informed signed consent was obtained from subjects donating 6 mL of peripheral blood.

Patients with SARS. The initial study included 309 patients with confirmed SARS recruited from 3 major hospitals that treated patients with SARS in Hong Kong during the 2003 outbreak, namely, Pamela Youde Nethersole Hospital, Princess Margaret Hospital, and United Christian Hospital. Subsequently, we further recruited 508 patients with SARS from SARS follow-up outpatient clinics in the 3 previously mentioned hospitals and 3 others, namely, Queen Mary Hospital, Alice Ho Miu Ling Nethersole Hospital, and Prince of Wales Hospital. All 817 patients with SARS were confirmed by serologic analysis and/or reverse-transcriptase polymerase chain reaction (PCR). Their clinical data were retrospectively obtained from Hospital Authority, Hong Kong, with permission from all attending clinicians of the respective hospitals. These included age; sex; length of hospital stay; treatment in an intensive care unit (ICU) and duration of ICU treatment; whether patients required assisted ventilation, steroid treatment, pulse steroids, or intravenous immunoglobulin (IVIG); and final outcome in terms of survival and death. Because most of the patients were recruited from the SARS follow-up clinics after discharge, the proportion of deaths was small and could not be used as a measure of outcome. Results of hematological and biochemical laboratory investigations on admission were also retrieved. These included hemoglobin level, absolute lymphocyte count,

Table 2. Clinical profile of patients with severe acute respiratory syndrome (SARS) recruited for study.

Characteristic	Initially recruited	All
Treated in ICU	59 (21.7)	136 (16.6)
Required ventilation	46 (16.9)	76 (9.3)
Received steroid treatment	258 (94.9)	795 (97.3)
Received pulse steroid/IVIG	163 (60.0)	517 (63.3)
Length of hospital stay, days		
Mean (SD)	33.2 (16.9)	28.23 (17.9)
Median (range)	28.0 (4–169)	23.0 (4–235)
Death	12 (4.4)	12 (1.5)
Length of ICU stay, days		
Mean (SD)	9.0 (17.1)	2.66 (10.8)
Median (range)	9.0 (1–80)	0 (0–139)

NOTE. Data are no. (%), unless otherwise indicated. ICU, intensive care unit; IVIG, intravenous immunoglobulin.

platelet count, WBC count, and biochemical indices of alanine aminotranseferase, albumin, globulin, creatinine kinase, LDH, urea, sodium, potassium, and serum creatinine.

Controls. Three control groups were recruited for this study. For our initial analysis, we recruited unaffected health care workers (HCWs) who had worked in SARS wards, who had worked in SARS ICU or cohort wards for patients with suspected SARS, and/or who had performed high-risk procedures. All 307 who had volunteered for the study remained disease free and were confirmed to be seronegative for SARS at the end of the outbreak. A simple self-administered questionnaire documented the type of wards they had worked in, the duration of work, and whether they had performed high-risk procedures such as ambu-bagging, nasopharyngeal suction, tracheal intubation, endotracheal suction, disposal of excreta, chest physiotherapy, and feeding of highly dependent patients. Subsequently, 2 further groups of controls were also recruited: outpatient control (OPC) subjects consisting of 290 individuals randomly recruited from the general outpatient clinics at least 2 months after the SARS outbreak with no clinical history or signs or symptoms of inflammation or infection and household contact control (HHC) subjects consisting of 309 household members of patients with SARS who remained unaffected and confirmed to be seronegative at the end of the outbreak. All HHC subjects recruited were asked to state their relationship with the patient(s) with SARS as well as with other family members in the household. To prevent genotype and allele frequency distribution bias, family members of the same household who were genetically related were taken into consideration in the statistical analysis of genotypes. All control subjects were Chinese from Hong Kong.

Genomic DNA from peripheral blood samples were extracted following conventional methods, adopting level 3 biosafety precautions in accordance with the World Health Organization and Centers for Disease Control and Prevention guidelines. The HHC subjects donated saliva samples for extraction of buccal DNA by Oragene DNA Self-Collection Kit (DNA Genotek).

Selection of polymorphisms for study. Nonsynonymous coding single nucleotide polymorphisms (nsSNPs) and 3′ untranslated region (3′UTR) SNPs of *ICAM3*, *FCER2*, *DC-SIGN*, and *L-SIGN* were identified from the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). We specifically looked for nsSNPs that may result in structural changes of the encoded protein leading to functional effect, as well as 3′UTR SNPs. At the time the study was initiated, no nsSNPs or 3′UTR SNPs were reported for *DC-SIGN*. Allele frequency of the SNPs was first evaluated in 90 unaffected healthy individuals, and only those with minor allele frequency >5% were further investigated. Five SNPs for *FCER2* and 1 for *ICAM3* were thus selected for study. The nsSNP for *L-SIGN* with minor allele frequency <5% was not further investigated.

Genotyping by Sequenom. Genotyping of the initial SARS samples (309 cases) and HCW control subjects was done by Sequenom. MassARRAY AssayDesign software (Sequenom) was used to design amplification and allele-specific extension. PCRs were performed in 384-well plate format, and the amplified products were treated with alkaline phosphatase. The final allele-specific base extension was performed and then treated with SpectroClean (Sequenom) resin. Extended products were dispensed onto SpectroCHIP (Sequenom), separated by Bruker Autoflex MALDI-TOF mass spectrometer (Brucker) and analyzed by SpectroTYPER (Sequenom). In every plate assayed, there was 1 well for blank control and 5 wells for duplicate check on 5 samples for quality control.

Genotyping of the additional SARS samples and HHC and OPC group samples for the *ICAM3 Gly143* SNP was performed by Allelic Discrimination TaqMan Assay (assay C_15974025_10;

Table 3. Relationship of household contacts with patients with severe acute respiratory syndrome.

Relationship	Contacts, no. (%)
Father, mother, son, daughter, brother, and sister	178 (57.7)
Grandfather, grandmother, aunt, uncle, nephew, niece, and cousin	10 (3.2)
Family member without blood relationship (e.g., husband, wife, son/daughter-in-law, brother/sister-in-law, stepfather, etc.)	102 (33.0)
Contacts with no family relationship (e.g., friend, domestic helper, etc.)	19 (6.1)
Total	309

Table 4. Genotype and allele frequencies of *ICAM3* and *FCER2* polymorphisms.

		Initially recruited patients with SARS			HCW control subjects				
Polymorphism	Genotype	No.	%	HWE *P*	No.	%	HWE *P*	OR (95% CI)	*P*
ICAM3									
rs2304237 (Asp143Gly)	TT/CT/CC (Asp/GlyAsp/Gly)	194/49/2	79.2/20/0.8	.566	265/26/6	89.2/8.8/2.0	3.55×10^{-6}	2.17 (1.35–3.52)[a]	**.001**
FCER2									
rs4804773 (Trp62Arg)	CC/TC/TT (Trp/ArgTrp/Arg)	213/34/2	85.5/13.7/0.8	.621	246/36/4	86.0/12.6/1.4	.055	…	.855
rs889182	TT/TC/CC	186/56/6	75.0/22.6/2.4	.472	154/55/3	72.6/25.9/1.4	.439	…	.092
rs1990975	GG/GA/AA	175/61/7	72.0/25.1/2.9	.551	151/55/3	72.2/26.3/1.4	.421	…	.103
rs2287868	GG/GA/AA	106/104/28	44.5/43.7/11.8	.747	89/103/16	42.8/49.5/7.7	.062	…	.732
rs2303112	AA/CA/CC	92/115/40	37.2/46.6/16.2	.687	62/117/32	29.4/55.5/15.2	.055	…	.064
	Allele								
ICAM3									
rs2304237 (Asp143Gly)	T/C (Asp/Gly)	437/53	89.2/10.8	…	556/38	93.6/6.4	…	1.775 (1.148–2.742)	**.009**
FCER2									
rs4804773 (Trp62Arg)	C/T (Trp/Arg)	460/38	92.4/7.6	…	528/44	92.3/7.7	…	…	.970
rs889182	T/C	428/68	86.3/13.7	…	363/61	85.6/14.4	…	…	.768
rs1990975	G/A	411/75	84.6/15.4	…	357/61	85.4/14.6	…	…	.725
rs2287868	G/A	316/160	66.4/33.6	…	281/135	67.5/32.5	…	…	.713
rs2303112	A/C	299/195	60.5/39.5	…	241/181	57.1/42.9	…	…	.295

NOTE. The total no. of cases successfully genotyped for each single nucleotide polymorphism varied, depending on the success call rate generated from the MassARRAY Analyzer (Sequenom). Bold type indicates statistically significant values. CI, confidence interval; HCW, health care worker; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; SARS, severe acute respiratory syndrome.

[a] OR was obtained by comparing combined Gly-allele carriers with homozygous Asp carriers.

Applied Biosystems). The assay was performed according to conditions as described elsewhere [22]. Each 96-well plate reaction contained 1 negative control, controls for each genotype that was examined by direct sequencing, and there were 8 replicated samples.

Risk association analysis. Genotype distributions of the patient group and the control groups were assessed using the χ^2 test using SPSS for Windows (version 13.0), and odds ratio (OR) and 95% confidence intervals (CIs) were used to measure strength of association. Hardy-Weinberg equilibrium (HWE) and pair-wise linkage disequilibrium (LD) analysis were calculated using the χ^2 test, taking $P < .05$ for the level of significance. Haplotype frequencies were estimated using Arlequin (version 2.00; Genetics and Biometry Laboratory, University of Geneva) based on expectation-maximization algorithm. The standardized LD parameter (D′) was evaluated by 2LD [23]. Because a significant proportion of the HHC subjects recruited were genetically related to each other, we also used logistic regression with the cluster and robust methods (STATA program; version 9; StataCorp) [24] to factor in genetic relations of all subjects.

Analysis for association with clinical outcome. The χ^2 test was used to test for possible association with nominal clinical outcome measures such as those requiring ICU care and/or ventilation, requiring pulse steroid and/or IVIG treatment. For the analysis of numerical variables such as length of hospital stay and length of ICU stay, and hematological and biochemical laboratory indices, each of these parameters was first analyzed by Student's *t* test to compare the mean values of patients having wild-type versus the variant genotype. Because of the variations in the reference ranges of biochemical indices used by different hospitals, some of the values, such as LDH, alanine aminotransferase, and creatinine kinase, were standardized by dividing the actual values by the upper limit of normal reference range. Parameters for which significant difference was obtained were further studied using the χ^2 test. To identify the appropriate cutoff value to partition the values into 2 groups, values were arranged in ascending order forming a curve that fits a polynomial trend line. The cutoff value was taken at the inflection of the curve or at the point when the values begin to change exponentially.

RESULTS

Demographic data. The demographic features of the patients with SARS and the various control groups are summarized in

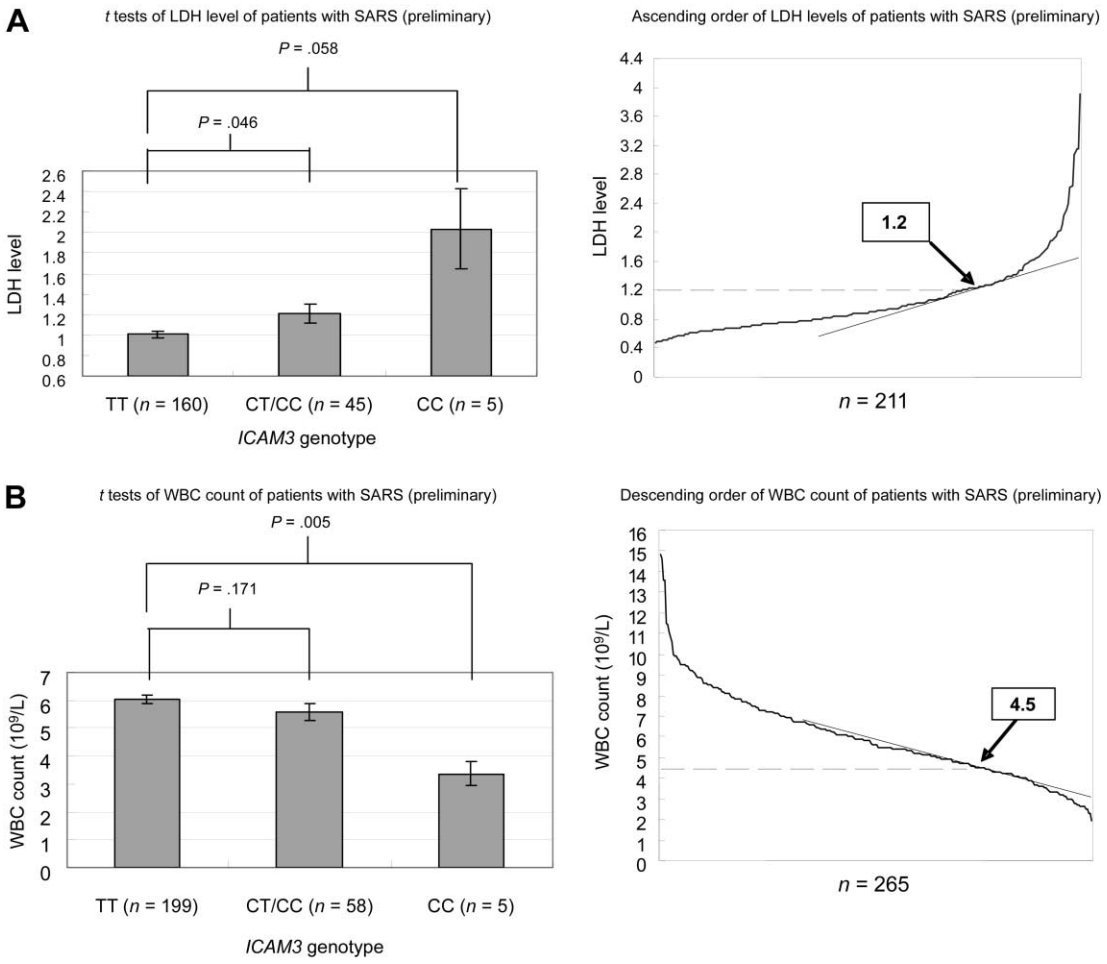

Figure 1. Preliminary studies. *A,* Genotype and allele frequencies of *ICAM3* of patients with severe acute respiratory syndrome (SARS) with normalized lactate dehydrogenase (LDH) levels on admission (as ratio to upper limit of normal reference range). *B,* Genotype and allele frequencies of *ICAM3* of patients with SARS with white blood cell (WBC) count on admission.

table 1. The clinical features are as summarized in table 2. In HCW control subjects, there were 269 (87.6%) who had worked in a SARS ward/SARS ICU, and the remaining 38 (12.4%) worked either in a suspected SARS cohort ward or in a general ward that had cared for patients with SARS. They had worked in these environments for 4–120 days (median, 50 [average, 52] days) during the SARS outbreak, and 178 (58%) had performed high-risk procedures. The 290 OPC subjects had previously been used in our genetic association study of L-SIGN for susceptibility to SARS [21]. However, only 260 cases had sufficient DNA left over for further analysis. The third control group of 309 HHC subjects was recontacted from the study of Leung et al. [2]. These consisted of asymptomatic close contact of patients with SARS who were serologically negative for SARS. Their relationship to the patients with SARS recruited is as summarized in table 3.

Genotype analysis of initial sample of 309 patients with SARS and 307 HCW controls. The genotype and allele frequencies of each of the polymorphisms studied on the initial cohort of 309 patients with SARS and 307 HCW controls are summarized in table 4. Genotypes were found to be in HWE for both the SARS and control populations for all *FCER2* and *ICAM3* SNPs, except for the *ICAM3 Asp143Gly* SNP (rs2304237) in the HCW population. This SNP was included in our statistical analysis for genetic association because it might be representing a preselected group with overrepresentation of protective genotype, accounting for Hardy-Weinberg disequilibrium. Genotype frequency analysis showed that the *ICAM3 Asp143Gly* SNPs were found to have significant risk association for susceptibility to SARS ($P = .001$, χ^2 test; OR, 2.17 [95% CI, 1.35–3.52]), with Gly-allele carriers being more at risk for SARS. Allelic association was also noted ($P = .009$, χ^2 test; OR,

Table 5. *ICAM3* genotype and allele analyses, with lactate dehydrogenase (LDH) levels on admission of patients with severe acute respiratory syndrome (preliminary).

ICAM3	Lower LDH level[a]	Higher LDH level[b]	OR (95% CI)	*P*[c]
Genotype[d]				
TT (Asp)	121 (81.7)	39 (68.4)	Reference	**.013**
CT (Gly/Asp)	26 (17.6)	14 (24.6)	…	.173
CC (Gly)	1 (0.7)	4 (7.0)	12.41 (1.35–114.40)	**.017**
C-carrier (Gly-carrier) (CT and CC)	27 (18.3)	18 (31.6)	2.07 (1.03–4.15)	**.039**
Allele[e]				
T (Asp)	268 (90.5)	92 (80.7)	Reference	…
C (Gly)	28 (9.5)	22 (19.3)	2.29 (1.25–4.20)	**.006**

NOTE. Data are no. (%), unless otherwise indicated. Bold type indicates statistically significant values. CI, confidence interval; OR, odds ratio.

[a] Normalized LDH levels on admission were less than the cutoff value (1.2).
[b] Normalized LDH level on admission were higher than the cutoff value (1.2).
[c] χ^2 test (3 × 2 table) for overall genotype.
[d] Lower LDH, $n = 148$; higher LDH, $n = 57$.
[e] Lower LDH, $n = 296$; higher LDH, $n = 114$.

1.775 [95% CI, 1.148–2.742]). None of the 5 *FCER2* SNPs studied showed significant association by genotype or allele frequency (table 4). The 4 3′UTR *FCER2* SNPs were found to be in LD with each other. On the other hand, *ICAM3* was not in LD with the *FCER2* SNPs. Haplotype analysis for *FCER2* showed no significant association in the haplotypes ($P > .05$, χ^2 test) (data not shown).

There was no significant association found for nominal clinical outcome measures such as those requiring ICU care and/or ventilation, requiring pulse steroid and/or IVIG treatment for all the *FCER2* and *ICAM3* SNPs studied. Other numeric clinical parameters showed no significant association for the 4 *FCER2* SNPs ($P > .05$, Student's *t* test). For the *ICAM3 Asp143Gly* SNP, however, borderline association for LDH level was observed ($P = .046$, comparing homozygous wild-type Asp versus Gly-allele carriers). An overall significant association ($P = .013$, χ^2 test) was observed; homozygous Gly versus homozygous Asp gave $P = .017$ (figure 1*A* and table 5). For WBC count, Student's *t* test gave $P = .005$ for homozygous Gly versus homozygous Asp. An overall significant association was found ($P = .007$, χ^2 test); homozygous Gly versus homozygous Asp gave $P = .012$ (χ^2 test). However, the number of cases with the homozygous Gly was too small for this significance to be valid (figure 1*B* and table 6). Moreover, all these values become nonsignificant after multiple testing correction for the number of laboratory indices analyzed ($n = 13$) (adjusted $P = .0039$).

Genotype analysis of additional 508 SARS samples and additional control groups for the* ICAM3 Asp143Gly *SNP. As the risk association of *ICAM3 Asp143Gly* SNP was based on genotyping data for which the control group deviated from HWE, we further tested for this association using other control groups. Although the OPC subjects may not necessarily have been exposed to SARS-CoV, the HHC subjects would have had similar chance of exposure to SARS-CoV as the HCW control subjects. Genotype data of both OPC and HHC groups were in HWE. However, no significant difference in genotype or allele frequency distribution was found between the initial 309 SARS samples with neither the HHC group nor the OPC group (data not shown). No significant difference was also found comparing these 2 control groups with the final SARS sample size of 817 cases (table 7). From this further analysis, it should be concluded there is no association of the *ICAM3 Asp143Gly* SNP with susceptibility to SARS infection.

The genotype data of the *ICAM3 Asp143Gly* SNP for the 817 SARS cases was further analyzed for possible association with LDH levels and WBC counts previously suggested in the initial SARS samples. Student's *t* test showed similar findings as that of the initial samples. The cutoff values for LDH levels for χ^2 test were 1.6, dividing the patient into low LDH and high LDH level groups, whereas that of WBC count was 4.5 (figure 2*A* and 2*B* and tables 8 and 9). For LDH level, the χ^2 test for overall genotype gave $P = .015$ and homozygous Gly versus homozygous Asp gave $P = .0067$ (OR, 4.31 [95% CI, 1.37–13.56]). Allelic association was also observed ($P = .0093$; OR, 1.75 [95% CI, 1.14–2.67]). All *P* values remained significant after multiple testing correction ($n = 2$) (adjusted $P = .025$), thus confirming the association for LDH level on this large

Table 6. *ICAM3* genotype and allele analyses, with white blood cell (WBC) counts on admission of patients with severe acute respiratory syndrome (preliminary).

ICAM3	Lower WBC count[a]	Higher WBC count[b]	OR (95% CI)	*P*[c]
Genotype[d]				
TT (Asp)	45 (68.2)	154 (80.6)	Reference	**.007**
CT (Gly Asp)	17 (25.8)	36 (18.9)	…	.155
CC (Gly)	4 (6.0)	1 (0.5)	0.073 (0.008–0.670)	**.012**
C-carrier (Gly-carrier)	21 (31.8)	37 (19.4)	0.515 (0.274–0.967)	**.037**
Allele[e]				
T (Asp)	107 (81.1)	344 (90.1)	Reference	…
C (Gly)	25 (18.9)	38 (9.9)	0.473 (0.273–0.819)	**.007**

NOTE. Data are no. (%), unless otherwise indicated. Bold type indicates statistically significant values. CI, confidence interval; OR, odds ratio.

[a] WBC count on admission less than the cutoff value (4.5).
[b] WBC count on admission higher than the cutoff value (4.5).
[c] χ^2 test (3 × 2 table) for overall genotype.
[d] Lower WBC count, $n = 66$; higher WBC count, $n = 191$.
[e] Lower WBC count, $n = 132$; higher WBC count, $n = 382$.

Table 7. Genotype and allele analysis of the *ICAM3* Asp143Gly, of all patients with severe acute respiratory syndrome (SARS) vs. outpatient control (OPC) subjects and vs. household contact control (HHC) subjects.

A. *ICAM3* genotype and allele frequencies.

	Patients with SARS		OPC subjects		
ICAM3	No. (%)	HWE *P*	No. (%)	HWE *P*	SARS vs. OPC *P*[a]
Genotype[b]		.378		.937	
TT (Asp)	534 (78.1)		206 (79.2)		.783
CT (GlyAsp)	138 (20.2)		51 (19.6)		.815
CC (Gly)	12 (1.8)		3 (1.2)		.771
C-carrier (Gly-carrier)	171 (20.9)		54 (20.8)		.699
Allele[c]					
T (Asp)	1206 (88.2)		463 (89.0)		Reference
C (Gly)	162 (11.8)		57 (11.0)		.594
			HHC subjects		SARS vs. HHC *P*[a]
Genotype[d]		.191		.809	
TT (Asp)	394 (77.4)		189 (80.1)		.581
CT (GlyAsp)	104 (20.4)		44 (18.6)		.531
CC (Gly)	11 (2.2)		3 (1.3)		.565
C-carrier (Gly-carrier)	115 (22.6)		47 (19.9)		.410
Allele[e]					
T (Asp)	892 (87.6)		422 (89.4)		Reference
C (Gly)	126 (12.4)		50 (10.6)		.321

B. *ICAM3* genotype analysis, taking into account the blood relationships.

SARS vs. HHC	OR (95% CI)	*P*
Overall genotypes TT/CT/CC comparison (Asp/GlyAsp/Gly)	1.10 (0.81–1.49)	.545
Non–C-carriers vs. C-carriers (non–Gly-carriers vs. Gly-carriers)	1.12 (0.80–1.56)	.526

NOTE. In part A, patients who were blood related were excluded from analysis; genotype χ^2 test was performed using TT (Asp) genotype or T (Asp) allele as reference. In part B, logistic regression model and robust cluster method were performed using STATA program (version 9). CI, confidence interval; OR, odds ratio.

[a] χ^2 test (3×2 table) for overall genotype.
[b] Patients with SARS, $n = 684$; OPC subjects, $n = 260$.
[c] Patients with SARS,$n = 1368$; OPC subjects, $n = 520$.
[d] Patients with SARS, $n = 509$; HHC subjects, $n = 236$.
[e] Patients with SARS, $n = 1018$; HHC subjects, $n = 472$.

sample size. Association for WBC count was also demonstrated for homozygous Gly versus homozygous Asp ($P = .022$; OR, 0.30 [95% CI, 0.10–0.89]), which remained significant after multiple testing correction (adjusted $P = .025$). It is important to note that the homozygous Gly genotype associated with higher LDH levels was associated on the other hand with lower WBC counts.

DISCUSSION

In this large genetic association study of SARS susceptibility, we compared the genotype of 817 patients with SARS with that of 3 groups of control subjects of 906 unaffected individuals. Although comparison of patients with SARS genotype with that of HCW control subjects suggested risk association for the *ICAM3 Asp143Gly* SNP, this association could not be confirmed on comparison with the other 2 control groups. The deviation from HWE in the HCW control group is intriguing as it was also observed for the *L-SIGN (CLEC4M)* tandem-neck repeats we had previously reported [21], for which genotype had been confirmed by Southern blot analysis. In that study, the association observed between SARS and HCW control subjects was confirmed by comparing patients with SARS with outpatient

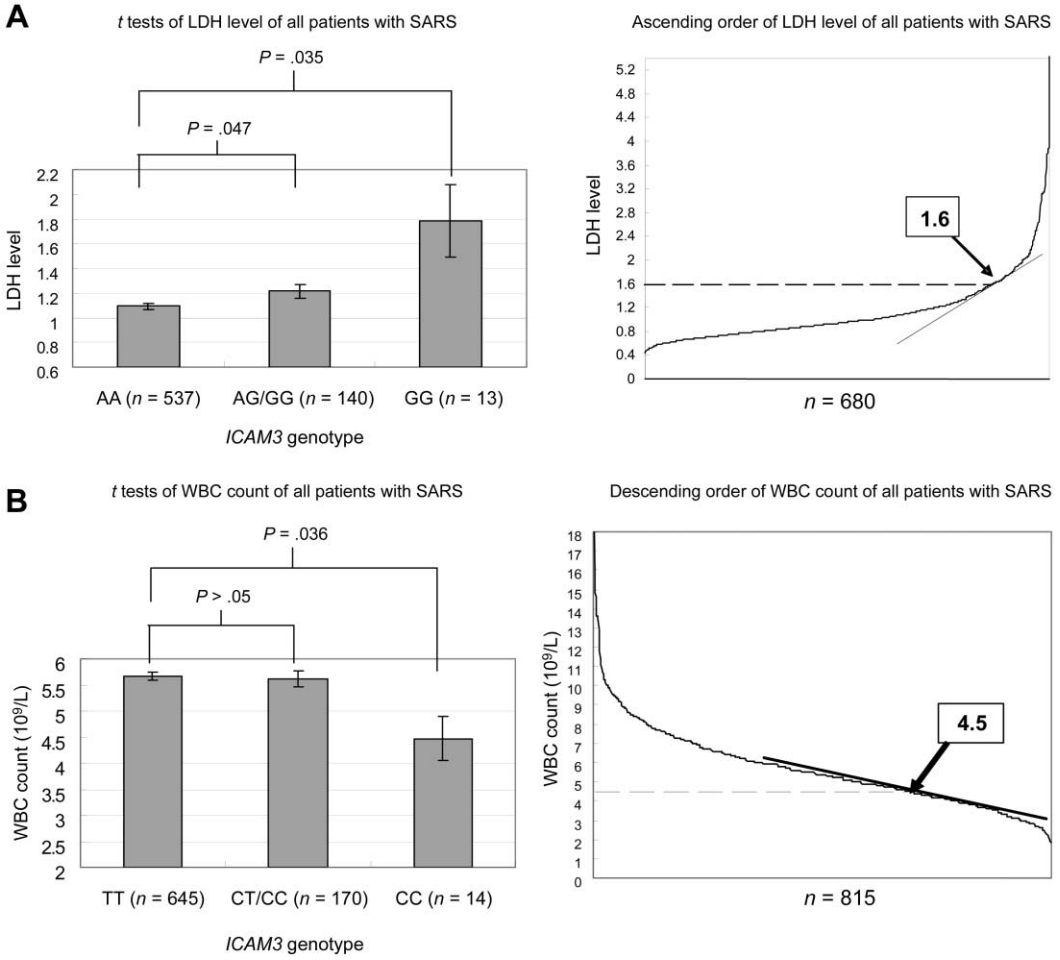

Figure 2. *A,* Genotype and allele frequencies of *ICAM3* of all patients with severe acute respiratory syndrome (SARS) with normalized lactate dehydrogenase (LDH) level on admission (as ratio to upper limit of normal reference range). *B,* Genotype and allele frequencies of *ICAM3* of all patients with SARS with white blood cell (WBC) counts on admission.

control and random control subjects, which both showed the same significant association. Because 22% of all patients with SARS in Hong Kong were HCWs [25], we therefore reckon that the unaffected health care workers recruited for study may likely represent a preselected group with overrepresentation of the protective L-SIGN genotype.

Repeated genotyping of 170 cases from the HCW control samples using Allelic Discrimination TaqMan Assay reconfirmed their *ICAM3* genotype, which still deviated from HWE ($P = 8.34 \times 10^{-4}$). Furthermore, χ^2 test analysis comparing the ICAM3 genotypes of the 817 patients with SARS with these 170 HCW control subjects continued to demonstrate significant difference ($P = .003$). In contrast, the genotype and allele frequency distribution of the OPC and HHC groups was very similar to that of the patients with SARS. It is worth noting that the age and sex distribution of both OPC and HHC groups (table 1) is better matched to that of the patients with SARS recruited than to that of the HCW control subjects. However, confounding factors such as age and sex had already been accounted for with the use of logistic regression analysis with adjustment for age and sex (data not shown). The presence of other confounding factors as yet unaccounted for in this HCW population probably gave rise to this apparent association. Thus, with the analysis of 3 control groups, no association of the *ICAM3 Asp143Gly* SNP with susceptibility to SARS infection can be concluded. On the other hand, genotype analysis of our 817 patients with SARS confirmed the association of higher LDH levels and lower WBC counts with the homozygous Gly143 genotype of *ICAM3*, which supports the role of *ICAM3* in the immunopathogenesis of SARS.

The polymorphism *ICAM3 Asp143Gly* involves the replacement of a large acidic amino acid aspartic acid by a small neutral amino-acid glycine in the extracellular domain. Although the protein structure of *ICAM3* is not yet disclosed, it is postulated to be similar to that of *ICAM1* [26], whose structure has been demonstrated [27]. The *ICAM3 Asp143Gly* SNP is located at

Table 8. Genotype and allele analysis of the *ICAM3* Asp143Gly, with lactate dehydrogenase (LDH) levels on admission of all patients with severe acute respiratory syndrome.

ICAM3	Lower LDH level[a]	Higher LDH level[b]	OR (95% CI)	*P*[c]
Genotype[d]				
TT (Asp)	469 (80.6)	68 (71.6)	Reference	**.015**
CT (GlyAsp)	105 (18)	22 (23.2)	…	.168
CC (Gly)	8 (1.4)	5 (5.2)	4.31 (1.37–13.56)	**.007**
C-carrier (Gly-carrier) (CT and CC)	113 (19.4)	27 (28.4)	1.65 (1.009–2.69)	**.044**
Allele[e]				
T (Asp)	1043 (89.6)	158 (83.2)	Reference	…
C (Gly)	121 (10.4)	32 (16.8)	1.75 (1.14–2.67)	**.009**

NOTE. Data are no. (%), unless otherwise indicated. Bold type indicates statistically significant values. CI, confidence interval; OR, odds ratio.

[a] Normalized LDH levels on admission were less than the cutoff value (1.6).
[b] Normalized LDH levels on admission were less than the cutoff value (1.6).
[c] χ^2 test (3×2 table) for overall genotype.
[d] Lower LDH, $n = 582$; higher LDH, $n = 95$.
[e] Lower LDH, $n = 1164$; higher LDH, $n = 190$.

the junction of domain 1 and 2 and the interchange of an acidic aspartic acid to a small neutral glycine at this junction is likely to affect the structuring of ICAM3 and subsequent dimer formation. Because DC-SIGN/ICAM3 receptor-ligand pair is crucial for antigen-presenting cells—for example, for DCs to form immunological synapse with naive T cells—the association of the *ICAM3 Asp143Gly* SNP with LDH levels and WBC counts found in our study suggest that the SNP may affect the interaction of T cells with DCs, thereby modulating T cell response in the immunopathogenesis of SARS. We therefore postulate that this conformational change may affect the binding capacity of the receptor, thus affecting lymphocyte activation and the initiation of immune response. Further experiments are required to examine this possibility.

The results of this study suggest that, although *FCER2* and *ICAM3* SNPs are not associated with susceptibility to SARS-CoV infection, the *ICAM3 Asp143Gly* SNP is associated with LDH levels and lower WBC counts in patients with SARS on admission. These findings are in keeping with the role of ICAM3 in T cell activation and the immune response. Association with susceptibility to SARS infection, on the other hand, would more likely to be found with binding receptors of the virus, such as L-SIGN, as reported by our group [21]. The absence of significant association of *ICAM3 Asp143Gly* with other clinical parameters is likely related to the fact that the patients with SARS recruited for our study came from 6 different hospitals throughout Hong Kong. This may have introduced confounding factors that are difficult to eliminate, such as possible differences in management preferences relating to the length of hospital stay, ICU care, and decision to initiate assisted ventilation and administration of steroids. On the other hand, laboratory parameters were easier to standardize. Other parameters reported to correlate with clinical outcome such as viral load and chest x-ray appearances were only available for a relatively small number of patients.

Although it is generally understood that LDH levels are nonspecific reflections of tissue destruction, the finding of associated higher LDH levels with lower WBC counts suggests that raised LDH levels in patients with SARS may also be the result of leukocyte destruction associated with immune response. The finding of decreased peripheral T, B, and NK cells and high levels of plasma proinflammatory cytokines in patients with SARS lend support to this [28, 29]. An increase in LDH levels in the acute phase of SARS infection has been postulated to be possibly related to immune hyperactivity [30]. High peak LDH levels have been reported to be an independent predictor of adverse outcome [31–34]. Thus patients with SARS who are homozygous for CC genotype of the *ICAM3 Asp143Gly* SNP are associated with a 4-fold chance of higher LDH levels on admission and poorer prognosis. Although the SARS outbreak appears to have been contained, the molecular determinants and pathogenesis of SARS remain unclear. Functional studies to investigate the role of *ICAM3 Asp143Gly* polymorphism in influencing the initiation of immune response to SARS-CoV infection will contribute toward a better understanding of the pathogenesis of SARS.

Table 9. Genotype and allele analysis of the *ICAM3* Asp143Gly, with white blood cell (WBC) counts of all patients with severe acute respiratory syndrome.

ICAM3	Lower WBC count[a]	Higher WBC count[b]	OR (95% CI)	*P*[c]
Genotype[d]				
TT (Asp)	186 (75.6)	459 (80.7)	Reference	**.047**
CT (GlyAsp)	52 (21.1)	104 (18.3)	…	.270
CC (Gly)	8 (3.3)	6 (1.0)	0.30 (0.10–0.89)	**.022**
C-carrier (Gly-carrier) (CT and CC)	60 (24.4)	110 (19.3)	…	.102
Allele[e]				
T (Asp)	424 (86.2)	1022 (89.8)	Reference	…
C (Gly)	68 (13.8)	116 (10.2)	0.71 (0.51–0.98)	**.034**

NOTE. Data are no. (%), unless otherwise indicated. Bold type indicates statistical significant values. CI, confidence interval; OR, odds ratio.

[a] WBC count on admission less than the cutoff value (4.5).
[b] WBC count on admission higher than the cutoff value (4.5).
[c] χ^2 test (3×2 table) for overall genotype.
[d] Lower WBC, $n = 246$; higher WBC, $n = 569$.
[e] Lower WBC, $n = 492$; higher WBC, $n = 1138$.

Acknowledgments

We are indebted to Prof. Lap-Chee Tsui for his invaluable advice and support for this project. We thank Ann Yip, Bobo Wong, and N. C. Chan for their assistance in collecting blood samples from the health care workers; Phoebe Ng, Crystal Lai, and Pui Yee Fong (Genome Research Centre) and Kelvin Koo, Vicky Tin, and W. C. Xue (Department of Pathology) for their technical support; and Dr. K. H. Chan (Department of Microbiology, University of Hong Kong) for performing severe acute respiratory syndrome (SARS) serologic testing on health care workers. We also thank Dr. Vivian Wong and Ms. Edwina Shung (Hospital Authority SARS Collaborative Group) for the retrieval of clinical data of patients with SARS from the central database.

References

1. Tsang KW, Ho PL, Ooi GC, et al. A cluster of cases of severe acute respiratory syndrome in Hong Kong. N Engl J Med **2003**; 348:1977–85.
2. Leung GM, Chung PH, Tsang T, et al. SARS-CoV antibody prevalence in all Hong Kong patient contacts. Emerg Infect Dis **2004**; 10:1653–6.
3. Morris JG Jr, Potter M. Emergence of new pathogens as a function of changes in host susceptibility. Emerg Infect Dis **1997**; 3:433–41.
4. Lin M, Tseng HK, Trejaut JA, et al. Association of HLA class I with severe acute respiratory syndrome coronavirus infection. BMC Med Genet **2003**; 4:9.
5. Ng MH, Lau KM, Li L, et al. Association of human-leukocyte-antigen class I (B*0703) and class II (DRB1*0301) genotypes with susceptibility and resistance to the development of severe acute respiratory syndrome. J Infect Dis **2004**; 190:515–8.
6. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature **2003**; 426: 450–4.
7. Chiu RW, Tang NL, Hui DS, et al. ACE2 gene polymorphisms do not affect outcome of severe acute respiratory syndrome. Clin Chem **2004**; 50:1683–6.
8. Marzi A, Gramberg T, Simmons G, et al. DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. J Virol **2004**; 78:12090–5.
9. Yang ZY, Huang Y, Ganesh L, et al. pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J Virol **2004**; 78:5642–50.
10. Jeffers SA, Tusell SM, Gillim-Ross L, et al. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci USA **2004**; 101:15748–53.
11. Soilleux EJ, Barten R, Trowsdale J. DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13. J Immunol **2000**; 165: 2937–42.
12. Bashirova AA, Geijtenbeek TB, van Duijnhoven GC, et al. A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. J Exp Med **2001**; 193:671–8.
13. Geijtenbeek TB, Torensma R, van Vliet SJ, et al. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell **2000**; 100:575–85.
14. Fawcett J, Holness CL, Needham LA, et al. Molecular cloning of ICAM-3, a third ligand for LFA-1, constitutively expressed on resting leukocytes. Nature **1992**; 360:481–4.
15. Montoya MC, Sancho D, Bonello G, et al. Role of ICAM-3 in the initial interaction of T lymphocytes and APCs. Nat Immunol **2002**; 3: 159–68.
16. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature **1998**; 392:245–52.
17. Dustin ML. Role of adhesion molecules in activation signaling in T lymphocytes. J Clin Immunol **2001**; 21:258–63.
18. Bleijs DA, Geijtenbeek TB, Figdor CG, van Kooyk Y. DC-SIGN and LFA-1: a battle for ligand. Trends Immunol **2001**; 22:457–63.
19. van Kooyk Y, Geijtenbeek TB. A novel adhesion pathway that regulates dendritic cell trafficking and T cell interactions. Immunol Rev **2002**; 186:47–56.
20. Galea P, Vermot-Desroches C, Le CC, Wijdenes J, Chermann JC. Circulating cell adhesion molecules in HIV1-infected patients as indicator markers for AIDS progression. Res Immunol **1997**; 148:109–17.
21. Chan VS, Chan KY, Chen Y, et al. Homozygous L-SIGN (CLEC4M) plays a protective role in SARS coronavirus infection. Nat Genet **2006**; 38:38–46.
22. Gold B, Kalush F, Bergeron J, et al. Estrogen receptor genotypes and haplotypes associated with breast cancer risk. Cancer Res **2004**; 64: 8891–900.
23. Zhao JH. 2LD, GENECOUNTING and HAP: computer programs for linkage disequilibrium analysis. Bioinformatics **2004**; 20:1325–6.
24. Boston RC, Sumner AE. STATA: a statistical analysis system for examining biomedical data. Adv Exp Med Biol **2003**; 537:353–69.
25. Chan-Yeung M. Severe acute respiratory syndrome (SARS) and healthcare workers. Int J Occup Environ Health **2004**; 10:421–7.
26. Song G, Yang Y, Liu JH, et al. An atomic resolution view of ICAM recognition in a complex between the binding domains of ICAM-3 and integrin alphaLbeta2. Proc Natl Acad Sci USA **2005**; 102:3366–71.
27. Yang Y, Jun CD, Liu JH, et al. Structural basis for dimerization of ICAM-1 on the cell surface. Mol Cell **2004**; 14:269–76.
28. Jones BM, Ma ES, Peiris JS, et al. Prolonged disturbances of in vitro cytokine production in patients with severe acute respiratory syndrome (SARS) treated with ribavirin and steroids. Clin Exp Immunol **2004**; 135:467–73.
29. Cui W, Fan Y, Wu W, Zhang F, Wang JY, Ni AP. Expression of lymphocytes and lymphocyte subsets in patients with severe acute respiratory syndrome. Clin Infect Dis **2003**; 37:857–9.
30. Chan MH, Wong VW, Wong CK, et al. Serum LD1 isoenzyme and blood lymphocyte subsets as prognostic indicators for severe acute respiratory syndrome. J Intern Med **2004**; 255:512–8.
31. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med **2003**; 348:1986–94.
32. Leong HN, Earnest A, Lim HH, et al. SARS in Singapore—predictors of disease severity. Ann Acad Med Singapore **2006**; 35:326–31.
33. Chang HL, Chen KT, Lai SK, et al. Hematological and biochemical factors predicting SARS fatality in Taiwan. J Formos Med Assoc **2006**; 105:439–50.
34. Choi KW, Chau TN, Tsang O, et al. Outcomes and prognostic factors in 267 patients with severe acute respiratory syndrome in Hong Kong. Ann Intern Med **2003**; 139:715–23.