Mannose-Binding Lectin in Severe Acute Respiratory Syndrome Coronavirus Infection

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Little is known about the innate immune response to severe acute respiratory syndrome (SARS) coronavirus (CoV) infection. Mannose-binding lectin (MBL), a key molecule in innate immunity, functions as an ante-antibody before the specific antibody response. Here, we describe a case-control study that included 569 patients with SARS and 1188 control subjects and used in vitro assays to investigate the role that MBL plays in SARS-CoV infection. The distribution of *MBL* gene polymorphisms was significantly different between patients with SARS and control subjects, with a higher frequency of haplotypes associated with low or deficient serum levels of MBL in patients with SARS than in control subjects. Serum levels of MBL were also significantly lower in patients with SARS than in control subjects. There was, however, no association between *MBL* genotypes, which are associated with low or deficient serum levels of MBL, and mortality related to SARS. MBL could bind SARS-CoV in a dose- and calcium-dependent and mannan-inhibitable fashion in vitro, suggesting that binding is through the carbohydrate recognition domains of MBL. Furthermore, deposition of complement C4 on SARS-CoV was enhanced by MBL. Inhibition of the infectivity of SARS-CoV by MBL in fetal rhesus kidney cells (FRhK-4) was also observed. These results suggest that MBL contributes to the first-line host defense against SARS-CoV and that MBL deficiency is a susceptibility factor for acquisition of SARS.

Severe acute respiratory syndrome (SARS), a newly emerged infectious disease of humans, has affected >25 countries, with 8098 cases and 774 deaths reported to the World Health Organization (WHO) during the first half of 2003 [1]. The most affected locations were mainland China, Hong Kong, Taiwan, Singapore, and To-

ronto, Canada [2–5]. A novel virus, the SARS coronavirus (SARS-CoV), was identified as the etiological agent [6–8] and was subsequently confirmed by fulfillment of Koch's postulates [9]. The genome of SARS-CoV is sufficiently different from all previously known coronaviruses to warrant its designation as a new coronavirus group [10, 11]. The surface spike (S) protein is a major target of the host immune response to coronaviruses and plays an important role in the attachment and fusion of the virus to host cells. Postgenomic characterization of SARS-CoV has revealed that the glycosylation of this surface S protein is a high-mannose structure [12], but knowledge of the pathogenesis of and immune responses to SARS-CoV is still limited.

Mannose-binding lectin (MBL) is a key pattern-recognition molecule in innate immunity and functions as an ante-antibody, a humoral factor that plays a critical role in the first-line host defense before the production of antibodies [13]. MBL binds, through its mul-

tiple carbohydrate recognition domains (CRDs), to repeating arrays of carbohydrate structures on microbial surfaces [14], thereby activating the complement system via MBL-associated serine protease (MASP)–1 and –2 [15, 16] (also known as "the lectin pathway") or enhancing phagocytosis by acting as an opsonin [17–19]. Single-nucleotide polymorphisms (SNPs) in the promoter and coding regions of the *MBL* gene *(mbl2)* have functional effects on serum levels of MBL [20–22]. MBL deficiency increases the generalized susceptibility of an individual to infectious diseases [23, 24]. Increased susceptibility to infection by specific pathogens—including HIV, *Cryptosporidium parvum,* and *Neisseria meningitidis*—in MBL-deficient individuals has also been established [25–27]. MBL has also been shown to opsonize and inhibit the infectivity of influenza A virus [28].

In the present study, we examined the role that MBL plays in SARS-CoV infection. Our findings demonstrate that MBL-deficient individuals are more susceptible to SARS. Furthermore, MBL, through its CRDs, could bind SARS-CoV, leading to inhibition of infectivity independent of complement, although activation of complement via the lectin pathway was observed.

PATIENTS, MATERIALS, AND METHODS

Patients and samples. Approval for the patient study was granted by the Clinical Research Ethics Committee of the Institutional Review Board of The University of Hong Kong/ Hospital Authority Hong Kong West Cluster. We included in the study 569 Hong Kong Chinese patients with SARS who were admitted to 5 hospitals in Hong Kong between 10 March and 26 April 2003. These 569 patients represented 59.0% of the total 965 patients with SARS who were admitted to these 5 hospitals during the above period. The male to female ratio was similar between the 569 study patients (249:320) and the whole cohort (410:555), and the mean (SD) age of the study patients (41.2 [17.2] years) was similar to that of the whole cohort (40.7 [17.8] years). The patients fulfilled the modified WHO definition of SARS [29]. Either SARS-CoV antibody seroconversion or detection of SARS-CoV RNA in respiratory secretions by real-time quantitative polymerase chain reaction (PCR) was documented for at least 95% of the patients. The SARS-CoV antibody and RNA test results were obtained from the Government Virus Unit, Department of Health, Hong Kong Government, and the Department of Microbiology, The University of Hong Kong. The demographic features and outcomes of the patients were obtained from the eSARS database, which is maintained by the Hospital Authority SARS Collaborative Study Group. EDTA whole blood and serum samples—which, after investigations, were archived in the departments of pathology of Pamela Nethersole Youde Hospital, Princess Margaret Hospital, Queen Mary Hospital, and United Christian Hospital—were obtained from the 569 patients with SARS for genotyping and MBL assay.

A population of Hong Kong Chinese comprising 1188 individuals who had either been recruited from the local community or were healthy Red Cross blood donors served as control subjects. Their mean ± SD age was 19.4 ± 11.6 years; 658 were males, and 530 were females.

DNA was extracted from 200 μL of whole blood by use of the DNA Blood Mini Kit (Qiagen), in accordance with the manufacturer's instructions, and was stored at −20°C until further use. Separated serum was frozen at −70°C until further use.

Purification of MBL. MBL was purified from ethanol-fractionated human plasma paste (donated by C. Dash, Blood Products Laboratory, Elstree, UK), as described elsewhere [19]. Briefly, MBL was prepared by 2-step mannan-agarose affinity purification, followed by positive removal of trace immunoglobulin impurities. MBL prepared in this manner is noncovalently associated with MASP [15]. In addition, plasma-derived MBL prepared by the State Serum Institute (Copenhagen, Denmark; donated by C. Koch) was also used in some experiments and gave identical results.

Cells and virus. The fetal rhesus kidney cell line (FRhK-4) (American Type Culture Collection) was grown in MEM (Gibco-BRL) containing 10% heat-inactivated fetal calf serum (FCS) (Gibco-BRL), at 37°C in 5% CO_2. The SARS-CoV strain used in the present study was HKU39849 [6]. The $TCID_{50}$ of the virus was determined in microtiter plates in accordance with the Reed-Muench method. Briefly, 100 μL of serial 10-fold dilutions of virus were tested in quadruplicate on confluent layers of FRhK-4 cells grown on 96-well tissue-culture plates (Corning-Costar), and any cytopathic effect (CPE) was recorded after 2–3 days.

MBL genotyping and haplotyping. The SNPs in the promoter (−221 *X/Y*) and exon 1 (codon 54 *A/B*) of the *MBL* gene were genotyped by use of 2 separate cycling reactions of the TaqMan allele-discrimination system (Applied Biosystems), as described in our previous study [30]. The *MBL* mutant allele *B* is in linkage disequilibrium with the promoter polymorphism *X/Y*; therefore, *B* occurs only with *Y* [22]. To date, no exceptions to this association have been reported, and, accordingly, the data from the 2 separate TaqMan PCR reactions were combined to give 3 haplotypes: *YA, YB,* and *XA.* Therefore, 6 genotypes were identified in the present study: *YA/YA, YA/XA, XA/XA, YA/YB, XA/YB,* and *YB/YB. YB* is 1 of 3 mutant haplotypes commonly referred to as "*O*" [31, 32]. The other 2 known mutant haplotypes carrying structural polymorphisms, *C* (codon 57) and *D* (codon 52), were not tested for in the present study, since they are present at an extremely low frequency and probably were absent in the Chinese population [20, 21]. The frequencies of 2-locus haplotypes composed of the promoter and a structural allele were also estimated by performing the haplotype procedure of SAS/Genetic Software (version 8.2; SAS Institute), as described elsewhere [33]; the expectation-maximization algorithm was used to generate maximum-likelihood

Variable	Patients with SARS (n = 569)	Control subjects (n = 1188)	χ^2 test P	χ^2 test OR (95%CI)	Multiple logistic regression analysis P^a	OR (95% CI)
MBL genotype			<.001		.016	
YA/YA	211 (37.1)	530 (44.6)	…	…	…	…[b]
YA/XA	148 (26.0)	311 (26.2)	…	…	…	1.17 (0.88– 1.56)
XA/XA	20 (3.5)	53 (4.5)	…	…	…	0.94 (0.52– 1.73)
YA/YB	125 (22.0)	207 (17.4)	…	…	…	1.53 (1.12– 2.10)
XA/YB	49 (8.6)	68 (5.7)	…	…	…	1.81 (1.14– 2.88)
YB/YB	16 (2.8)	19 (1.6)	…	…	…	2.21 (0.98– 4.97)
YB carrier	190 (33.4)	294 (24.7)	<.001	1.52 (1.22–1.90)	<.001	1.55 (1.21–1.99)
MBL haplotype			<.001		.001	
YA	695 (61.1)	1578 (66.4)	…	…	…	…[b]
XA	237 (20.8)	485 (20.4)	…	…	…	1.10 (0.89– 1.35)
YB	206 (18.1)	313 (13.1)	…	…	…	1.51 (1.21– 1.90)

NOTE. Data are no. (%), unless otherwise noted. CI, confidence interval; OR, odds ratio. $P < .05$ was considered to be significant.

[a] P values were determined after adjustment for sex and mean age in patients with SARS vs. those in control subjects, by use of multiple logistic regression analysis.
[b] Reference group.

estimates of haplotype frequencies, given that genetic-marker genotypes are under the assumption of Hardy-Weinberg Equilibrium (HWE).

MBL assay. Serum levels of MBL were determined by use of an ELISA in which a mouse monoclonal anti–human MBL antibody (HYB 131-01; Antibody Shop), either unlabeled or labeled with biotin, was used as the primary or secondary antibody, respectively, as described elsewhere [34]. Horseradish-peroxidase (HRP)–conjugated streptavidin and substrate solution containing tetramethylbenzidine (R&D) were used for detection of bound secondary antibody, in accordance with the manufacturer's instructions.

Binding of MBL to SARS-CoV. Ninety-six-well flat-bottom polystyrene plates (Corning-Costar) were coated with either 100 μL (per well) of 10^3, 10^4, or 10^5 TCID$_{50}$ of SARS-CoV or 1% (wt/vol) bovine serum albumin (BSA) diluted in PBS. After incubation overnight at 4°C, wells were blocked for 1 h at room temperature with 1% BSA in PBS containing 0.05% NaN$_3$, were washed with Hanks' balanced salt solution (HBSS; Gibco-BRL) containing 0.05% Tween 20 (HBSS/tw), and were incubated for 2 h with 100 μL (per well) of 0, 1, 5, or 10 μg/mL MBL diluted in HBSS containing 20 mmol/L CaCl$_2$ (HBSS/Ca^{2+}). In some experiments, wells were incubated with 100 μL of MBL diluted in either HBSS containing 20 mmol/L EDTA or HBSS/Ca^{2+} containing 2 mg/mL mannan from *Saccharomyces cerevisiae* (Sigma-Aldrich). The plates were washed with HBSS/tw and were incubated for 1.5 h at room temperature with 0.2 μg/mL biotinylated monoclonal anti-MBL antibody (HYB 131-01; Antibody Shop) diluted in HBSS/Ca^{2+} containing 0.2% BSA. Bound antibody was detected by use of HRP-conjugated strep-

tavidin and tetramethylbenzidine substrate solution (R&D), in accordance with the manufacturer's instructions. The binding of MBL to immobilized SARS-CoV was evaluated by use of absolute absorbance values measured at 450 nm (A$_{450}$).
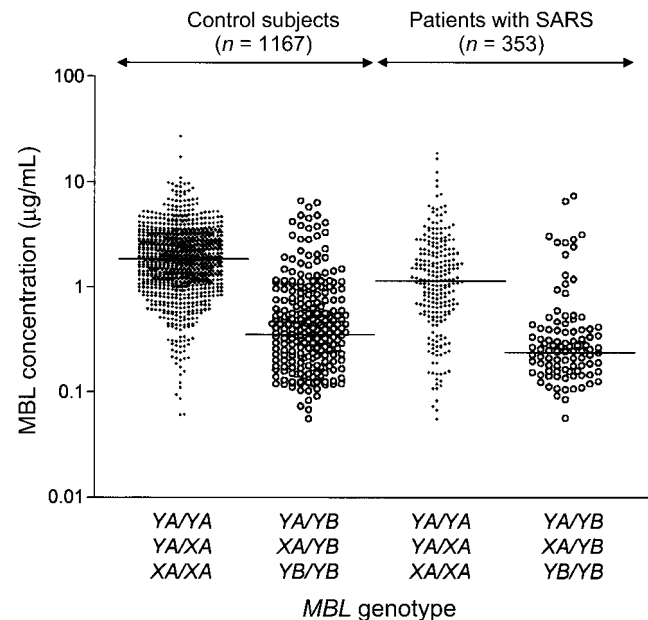

Figure 1. Serum levels of mannose-binding lectin (MBL), for *MBL* genotypes with or without the mutant allele *B*, in patients with severe acute respiratory syndrome (SARS), 19–23 days after the onset of disease. The horizontal bars indicate the medians for each genotype subgroup. Individuals with undetectable serum levels of MBL (<0.053 μg/mL) are not shown.
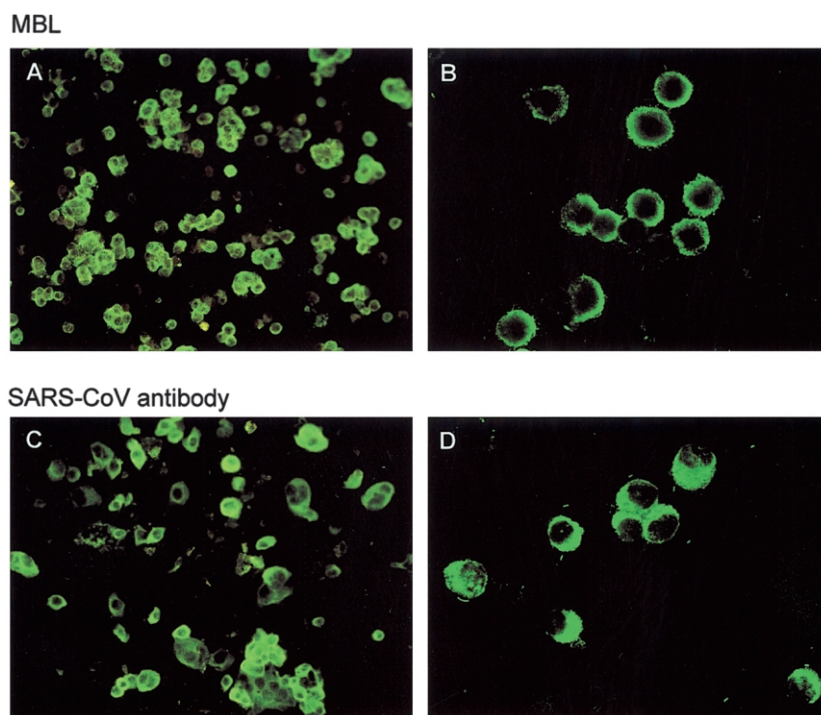
MBL

SARS-CoV antibody

Figure 2. Binding of mannose-binding lectin (MBL) to severe acute respiratory syndrome (SARS) coronavirus (CoV)–infected FRhK-4 cells. Binding of MBL to SARS-CoV–infected FRhK-4 cells was observed (*A* and *B*). Convalescent serum samples from patients with SARS with confirmed SARS-CoV antibody seroconversion were also tested, and binding of specific IgG to SARS-CoV–infected FRhK-4 cells was detected (*C* and *D*). Both MBL and anti–SARS-CoV antibody from serum samples from patients with SARS did not bind uninfected FRhK-4 cells (data not shown). Confocal imaging showed that the binding by MBL mostly occurred on the surface of the infected cell *(B)* and that the binding by specific IgG from serum samples from patients with SARS occurred on both the surface and in the cytoplasm of the infected cell *(D)*. Original magnifications, ×200 (*A* and *C*) and ×600 (*B* and *D*).

Deposition of complement C4 by the MBL pathway of complement activation. Ninety-six-well flat-bottom polystyrene plates were coated with either 100 μL (per well) of 10^5 TCID$_{50}$ of SARS-CoV or 1% BSA diluted in PBS. After incubation overnight at 4°C, wells were blocked for 1 h at room temperature with 1% BSA diluted in PBS containing 0.05% NaN$_3$, were washed 3 times with PBS containing 0.05% Tween 20 (PBS/tw), and were incubated with 100 μL (per well) of 0.16, 0.31, 0.625, 1.25, 2.5, or 5 μg/mL MBL diluted in 20 mmol/L Tris-HCl, 10 mmol/L CaCl$_2$, 1 mol/L NaCl, 0.05% (vol/vol) Triton X-100, and 0.1% BSA (pH 7.4). The wells that received no MBL (buffer only) were used as negative controls. After incubation overnight at 4°C, the wells were washed as described above and were incubated for 1.5 h at 37°C with 100 μL (per well) of 4 μg/mL purified human complement component C4 (Quidel) diluted in 4 mmol/L barbital, 145 mmol/L NaCl, 2 mmol/L CaCl$_2$, 1 mmol/L MgCl$_2$, and 1.5 mmol/L NaN$_3$ (pH 7.5). The wells were washed 3 times with PBS/tw, followed by incubation for 1.5 h at room temperature with mouse monoclonal anti-C4 antibody (4 μg/mL; Quidel) diluted in PBS/tw. The wells were washed as described above and were incubated for 1 h at room temperature with 100 μL (per well) of HRP–

anti-mouse IgG (Dako) diluted 1:1000 in PBS containing 0.2% BSA. The wells were washed and developed with tetramethylbenzidine substrate solution (R&D), as described above. The deposition of complement C4 on SARS-CoV by the MBL pathway was evaluated at A$_{450}$, according to the following formula: relative percentage of C4 deposition = (A$_{450}$ − A$_{450}$ of negative controls)/(A$_{450}$ of negative controls) × 100.

Inhibition of the infectivity of SARS-CoV by MBL. SARS-CoV (100 TCID$_{50}$) was treated for 1 h at 37°C with 0, 0.6, 1.3, 2.5, 5, 10, or 20 μg/mL MBL diluted in 100 μL of MEM. Treated virus was added to confluent layers of FRhK-4 cells grown in 96-well plates (Falcon; Becton Dickinson). After 1.5 h of absorption, the wells were washed twice with MEM and incubated with fresh MEM containing 1% FCS. A real-time quantitative PCR specific to the 1b gene region of SARS-CoV, with extraction of viral RNA from the culture supernatant after 24 h of incubation (as described elsewhere [35, 36]), was used to determine the infectivity of SARS-CoV in FRhK-4 cells. In some experiments, the percentage of culture cells producing CPE was determined by light microscopy after 48–60 h of incubation of infected cells; by 48 h, 100% CPE was observed in the cells infected with untreated SARS-CoV (100 TCID$_{50}$).
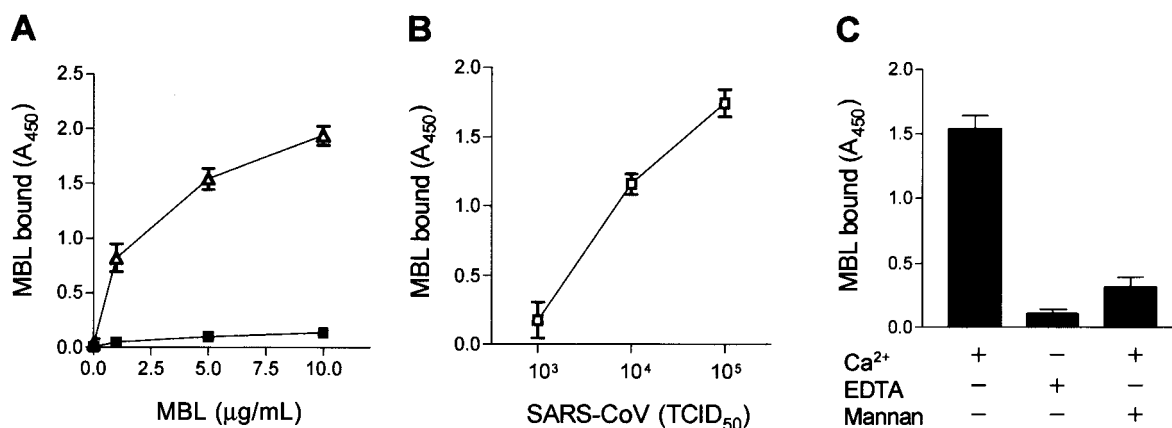
Figure 3. Binding of mannose-binding lectin (MBL) to severe acute respiratory syndrome (SARS) coronavirus (CoV). Binding of MBL to SARS-CoV was determined by use of a microtiter capture assay. Wells of microtiter plates were coated with SARS-CoV, and MBL was incubated for 2 h in the virus-coated wells. *A,* Amount of MBL. Incubation with increasing amounts of MBL resulted in increasing levels of detected MBL bound to immobilized SARS-CoV (Δ). In contrast, MBL binding did not increase when the wells were coated with bovine serum albumin (■). *B,* Amount of SARS-CoV. The amount of MBL binding was also dependent on the amount of SARS-CoV used to coat the wells. *C,* Presence of Ca^{2+}, EDTA, and/or mannan. To determine the nature of binding of MBL to SARS-CoV, MBL was incubated in wells coated with SARS-CoV in the presence of either Ca^{2+} or EDTA. The binding of MBL to SARS-CoV was reduced to background levels in the presence of EDTA, indicating that the interaction of MBL with SARS-CoV was Ca^{2+} dependent. Preincubation of MBL with 2 mg/mL mannan in the presence of Ca^{2+} also substantially reduced binding of MBL to SARS-CoV. These data provide evidence that MBL bound to immobilized SARS-CoV via its carbohydrate recognition domains. Results are presented as mean ± SE and are representative of 4 experiments.

Statistical analysis. Statistical analysis was performed by use of SAS software (version 6.12; SAS Institute). Comparisons of sex and *MBL* genotype distributions between patient and control groups or between subgroups of patients were performed by use of the χ^2 test. Age difference between the groups was analyzed by use of an unpaired *t* test. Serum levels of MBL in any 2 groups were compared by use of the Mann-Whitney *U* test. Multiple logistic regression analysis was used to investigate the association between SARS and *MBL* genotypes, after adjustment for differences in age and sex. Multiple linear regression analysis was also used to investigate factors that may determine serum levels of MBL: *MBL* genotype, age, sex, and disease status. A significance level of $P < .05$ was used for all analyses.

RESULTS

Association between genotypes associated with low serum levels of MBL and SARS. DNA from 569 patients with SARS (median [interquartile range {IQR}] age, 41.2 [29–51] years; 249 males and 320 females) and 1188 control subjects (median [IQR] age, 19.4 [10.2–28] years; 658 males and 530 females) was genotyped for *MBL* promoter *(X/Y)* and structural *(A/B)* polymorphisms (table 1). Three *MBL* haplotypes—*YA, XA,* and *YB*—were identified, where the mutant allele *B* is in linkage disequilibrium with the promoter alleles *X* and *Y* [22]. The distributions of the haplotypes in patients with SARS and in control subjects were in HWE ($P = .518$ and $P = .778$, respectively), and their frequencies were closely matched with

that estimated by haplotype analysis (data no shown), in which the estimated frequency of *XB* was as low as 0.00004 in both SARS patients and control subjects, suggesting that the mutant allele *B* is very unlikely to occur with the promoter allele *X*. There was a significant difference between patients with SARS and control subjects in terms of their *MBL* genotypes ($P < .001$) (table 1). Genotypes associated with low serum levels of MBL, with the mutant allele *B*, were significantly overrepresented in the patients with SARS. *MBL* haplotype frequencies also differed between patients with SARS and control subjects, and there was a significantly higher frequency of the haplotype associated with MBL deficiency, *YB* ($P < .001$). The overall carriage rate of *YB* was 33.4% in patients with SARS and 24.7% in control subjects ($P < .001$; odds ratio [OR], 1.52).

We subsequently analyzed carriage of the mutant allele *B* in patients with SARS who died ($n = 55$) and in those who were discharged from the hospital after recovery ($n = 514$). Carriage of *YB* did not differ between patients with SARS who recovered and those who died (34.1% [175/514] vs 29.1% [16/55]; $P = .56$).

Serum levels of MBL in patients with SARS. Serum levels of MBL in 353 patients with SARS, 19–23 days after the onset of disease, were determined. We found that the median serum level of MBL in these patients (0.733 [IQR, 0.263–1.796] µg/mL) was significantly lower than that in 1167 control subjects (1.369 [IQR, 0.572–2.598] µg/mL) ($P = .0004$); 13 patients with SARS and 63 control subjects had undetectable serum levels of MBL (<0.053 µg/mL). Multiple linear regression analy-
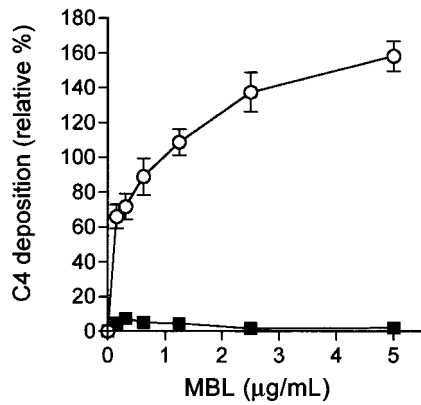
Figure 4. Mannose-binding lectin (MBL)–mediated deposition of complement C4 on severe acute respiratory syndrome (SARS) coronavirus (CoV). Different concentrations of MBL were incubated overnight at 4°C in microtiter plates coated with SARS-CoV. A consistent amount of C4 protein (4 μg/mL) was then incubated for 1.5 h at 37°C in the wells. Incubation with increasing amounts of MBL resulted in increasing deposition of complement C4 on SARS-CoV (○). In contrast, deposition of complement C4 did not increase when wells were coated with bovine serum albumin (■). Results are presented as mean ± SE and are representative of 2 experiments.

sis further revealed that *MBL* genotype and age were the significant factors that determined serum levels of MBL. The difference in serum levels of MBL between patients with SARS and control subjects was due to the significantly higher frequencies of genotypes with the mutant allele *B*, which was associated with low serum levels of MBL (figure 1), in the patients with SARS.

Binding of MBL to SARS-CoV and complement activation. MBL was found to bind to the surface of SARS-CoV–infected FRhK-4 cells as well as to immobilized SARS-CoV, by use of a microtiter capture assay (figures 2 and 3). The binding of MBL to SARS-CoV was calcium dependent and mannan inhibitable, suggesting that MBL, through its CRDs, can bind SARS-CoV. The binding was dose dependent for both MBL and SARS-CoV (figure 3). In addition, MBL was found to enhance deposition of complement C4 on immobilized SARS-CoV, suggesting that MBL activates complement on the virus via the lectin pathway (figure 4).

Inhibition of the infectivity of SARS-CoV by MBL. We further investigated the possible translation of binding of MBL to inhibition of the infectivity of SARS-CoV. SARS-CoV (100 $TCID_{50}$) preincubated in serum-free medium containing MBL at different concentrations (0.6–20 μg/mL) was added to confluent layers of FRhK-4 cells. After 24 h of incubation, the infectivity of SARS was determined by use of a real-time quantitative PCR specific to the 1b gene region of SARS-CoV, with extraction of viral RNA from the culture supernatant. MBL was found to have an inhibitory effect on SARS-CoV infection, in a dose-dependent fashion (figure 5). Such inhibition was also

observed in separate experiments that determined CPE in culture cells after 48–60 h of incubation; >75% inhibition of CPE was observed when SARS-CoV was pretreated with 5 or 10 μg/mL MBL, whereas partial inhibition of CPE (25%–50%) was observed when SARS-CoV was pretreated with 1.25 or 2.5 μg/mL MBL. No inhibition of CPE (i.e., 100% cells producing CPE) was found when SARS-CoV was pretreated with 0.6 μg/mL MBL, a concentration similar to the median serum level (0.52 μg/mL) in healthy individuals with group II MBL genotypes (e.g., *MBL B* carriers).

DISCUSSION

In the present study, we have shown that individuals either heterozygous or homozygous for *YB* had an OR of 1.52 for acquisition of SARS, and the frequency of *YB* was significantly higher in patients with SARS than in control subjects. Increasing ORs (1.53, 1.81, and 2.21) were indeed observed in the individuals with genotypes carrying 1 mutant haplotype *(YB)* with a haplotype associated with high serum levels of MBL *(YA)*, 1 mutant haplotype *(YB)* with a haplotype associated with low serum levels of MBL *(XA)*, and 2 mutant haplotypes *(YB)* (i.e., *YA/YB, XA/YB,* and *YB/YB,* respectively). Since *YB* is associated with low serum levels of MBL, we further established that patients with SARS had serum levels of MBL that were significantly lower than those in control subjects. We conclude, therefore, that low serum level of MBL is a susceptibility factor for acquisition of SARS; this conclusion further supports the role of MBL as a first line of defense in respiratory infections, as has been documented in many other clinical settings
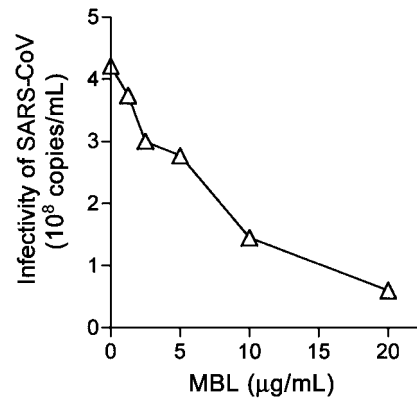

Figure 5. Inhibition of severe acute respiratory syndrome (SARS) coronavirus (CoV) infection. SARS-CoV pretreated with different concentrations of mannose-binding lectin (MBL) was added to confluent layers of FRhK-4 cells. After 1.5 h of absorption, the cells were washed and incubated with fresh medium. The infectivity of SARS-CoV was determined by real-time quantitative polymerase chain reaction specific to the 1b gene region of SARS-CoV, with extraction of viral RNA from the culture supernatant after 24 h of incubation. Increasing amounts of MBL resulted in decreasing the infectivity of SARS-CoV. Results are representative of 4 experiments.

[37–40]. For example, polymorphisms associated with low serum levels of MBL have been associated with frequency of respiratory tract infections in children [37], higher risk of invasive pneumococcal disease [38], greater mortality and impaired lung function related to cystic fibrosis [39], and more-frequent hospital admissions with respiratory tract infections related to chronic obstructive pulmonary disease [40]. Nevertheless, in the present study, *XA* (low-expression promoter) was not associated with susceptibility to SARS, possibly because the serum level of MBL in *XA* carriers was higher than that in *YB* carriers and was already sufficient to avert infection with SARS.

We also examined whether *YB* was associated with a greater risk of death in patients with SARS. The carriage of *YB,* however, did not differ between patients with SARS who died and patients with SARS who recovered. This lack of difference is likely due to the small number of the former who were tested; risk factors may not be easily detected in these patients, in particular in those with small effect size. It is also possible that, once an infection is established, many other factors contribute to the interaction between infectious agents and host defenses.

We attempted to investigate the role that MASP-2 plays in SARS-CoV infection, because MBL complexes with MASP-2 efficiently activate complement [18, 19]. A structural polymorphism (allele *A* or *G* in exon 3 of the *MASP-2* gene) that was recently found in the region encoding either a normal or a mutant form of the MASP-2 first domain CUB1 [41] was also genotyped in patients with SARS and control subjects (data no shown). However, we did not find the mutant allele *G,* which suggests that this allele, which is present in the Danish population at a gene frequency of 5%, is extremely rare in the Chinese population. Further experiments will be required to establish whether MASP-2 contributes to the immune response to SARS-CoV infection.

To elucidate the mechanisms underlying how MBL affects SARS-CoV infection, we investigated and demonstrated binding of and inhibition of the infectivity of SARS-CoV by MBL. MBL has been shown to function as an opsonin for other respiratory viruses, such as influenza A virus, and to inhibit hemagglutination and the infectivity of several strains of influenza A virus [28]. MBL inhibits hemagglutination and neutralizes the infectivity of influenza A virus by binding to the N-linked high-mannose carbohydrate side chain at the tip of the hemagglutinin S, thereby blocking the access of cell-surface receptors to the receptor-binding site on hemagglutinin [42]. Similarly, the S protein of SARS-CoV has 23 potential N-linked glycosylation sites [10], and some of the sites are glycosylated with high-mannose structures [12]. MBL selectively binds to mannose, glucose, L-fucose, N-acetylmannosamine, and N-acetylglucosamine, whereas galactose is not bound [43]. Since the binding of MBL to SARS-CoV could be inhibited by mannan and since this binding was Ca^{2+} dependent, it is likely that the binding of MBL to SARS-CoV is through the interaction of the CRDs of the MBL with the mannose side chains of the S protein of SARS-CoV.

In vitro inhibition of the infectivity of SARS-CoV by MBL was achieved at physiological levels. Since MBL may mediate complement activation, as demonstrated by our assay for in vitro deposition of complement C4, and modulate phagocytosis of SARS-CoV by macrophages, resulting in other antiviral responses, the effect of MBL on SARS-CoV in vivo may be more complex. Further experiments to delineate the relative roles between MBL, complement, and macrophages, in control of SARS-CoV infection, are required.

Accumulating evidence has shown that angiotensin-converting enzyme 2 (ACE2), a metallopeptidase, is a functional receptor for SARS-CoV that can bind to the S1 domain of the S protein of SARS-CoV [44–46]. Anti-ACE2 antibody can block viral replication in African green monkey kidney cells (Vero E6) [44, 46]. Furthermore, a recombinant human antibody against the S1 domain of the S protein of SARS-CoV has been shown to neutralize the virus on Vero E6 cells and inhibit formation of syncytia between cells expressing the S protein and those expressing ACE2 [47]. MBL is a serum C-type lectin, and, here, we have demonstrated that MBL binds SARS-CoV per se or infected cells and inhibits the infectivity of the virus on rhesus monkey kidney cells. These findings therefore support the possible role of MBL in the host defense against SARS-CoV infection by targeting the S protein of SARS-CoV before production of specific antibody. Taken together, our findings suggest that MBL-deficient individuals are more susceptible to SARS, and they provide insight into the possible mechanisms of the innate immune response to SARS-CoV infection. It will be important to assess the therapeutic and prophylactic effect of replenishment of MBL in MBL knockout animal models of SARS.

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