MAJOR ARTICLE

Association of Mannose-Binding Lectin Polymorphisms with Sepsis and Fatal Outcome, in Patients with Systemic Inflammatory Response Syndrome

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Genetic factors may predispose critically ill patients to increased risk of developing sepsis. Mannose-binding lectin (MBL) is an important factor in innate immune defense. We investigated whether MBL gene polymorphisms causing low levels of MBL are associated with the development and progression of sepsis in adult patients in intensive care units. In 272 prospectively monitored patients with systemic inflammatory response syndrome, different MBL genotypes were compared, with respect to microbiology, sepsis development, and survival. The presence of MBL variant alleles was associated with the development of sepsis, severe sepsis, and septic shock. An increased risk of fatal outcome was observed in patients carrying variant alleles. These data show that MBL insufficiency plays an important role in the susceptibility of critically ill patients to the development and progression of sepsis and confers a substantial risk of fatal outcome.

Systemic inflammatory response syndrome (SIRS) is associated with different overlapping scenarios, comprising invasive infection, dissemination of microbes secondary to injury, shock, and activation of inflammation by apparently noninfectious events [1]. SIRS can be self-limiting or can, in infected patients, progress to severe sepsis and septic shock [2]. Severe sepsis is a primary cause of death in intensive care units (ICUs) (responsible for 30%–50% of deaths) [3].

Failure of host defense mechanisms is likely to be involved in the variable presentation of SIRS and sepsis. Although the pathophysiology of SIRS and sepsis is very complex, it has been shown that genetic factors of importance for the inflammatory response—such as polymorphisms associated with regulation of the expression of tumor necrosis factor–α and interleukin-1 receptor antagonist gene—may be associated with susceptibility to and outcome of severe sepsis [4, 5]. In addition, genetic variation within the fibrinolytic system seems to be of importance for the outcome of sepsis [6–8].

Mannose-binding lectin (MBL) is a circulating liver-synthesized serum protein of importance for innate immune defense and is one of the recognition molecules in the lectin complement activation pathway (for a recent review, see [9]). Human MBL is derived from a single gene located on chromosome 10 *(mbl2)* [10, 11]. Interindividual differences in concentrations of MBL serum are mainly caused by structural variant alleles (*B, C,* and *D,* at codons 54, 57, and 52, respectively) in the *mbl2* gene [12–14]. The normal allele is named *A,* and the common designation for the variant alleles is *0.* In addition to the structural allele variants, differences in levels of MBL serum are determined by polymorphic sites in the promoter region of the *mbl2* gene [15, 16]. In particular, a base substitution at codon −221 (G→C; promoter allele *X*) is associated with low concentrations of MBL serum [15, 16].

MBL deficiency is associated with increased risk of

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Table 1. Primer and interpretation of mannose-binding lectin (MBL) polymerase chain reaction (PCR) sequence-specific priming.

Reaction	Name of allele	Primer		PCR product, bp	Haplotypes/interpretation						
		Forward	Reverse		*LXPA*	*LYPA*	*LYQA*	*HYPA*	*LYPB*	*LYQC*	*HYPD*
1	A non-B	AGTCGACCCAGATTGTAGGACAGAG	CCTTTTCTCCCTTGGTGC	277	x	x	x	x		w	x
2	B	GCAAAGATGGGCGTGATGA	GGGCTGGCAAGACAACTATTA	224					x		
3	A non-C	AGTCGACCCAGATTGTAGGACAGAG	CCTGGTTCCCCCTTTTCTC	287	x	x	x	x	x	x	x
4	C	AGTCGACCCAGATTGTAGGACAGAG	ACCTGGTTCCCCCTTTTCTT	288						x	
5	A non-D	AGTCGACCCAGATTGTAGGACAGAG	TCCCTTGGTGCCATCACG	270	x	x	x	x	x	x	x
6	D	AGTCGACCCAGATTGTAGGACAGAG	CTCCCTTGGTGCCATCACA	271							x
7	X	CATTTGTTCTCACTGCCACC	CTCAGGGAAGGTTAATCTCAG	285	x						
8	Y	CATTTGTTCTCACTGCCACG	CTCAGGGAAGGTTAATCTCAG	285		x	x	x	x	x	x
9	H	GGCTTAGACCTATGGGGCTA	GCTTCCCCTTGGTGTTTTAC	272				x			x
10	L	GGCTTAGACCTATGGGGCTA	GCTTCCCCTTGGTGTTTTAG	272	x	x	x		x	x	
11	P	TAGGACAGAGGGCATGCTC	AGGATCCAGGCAGTTTCCTCT-GGAAGG	334	x	x		x	x		x
12	Q	TAGGACAGAGGGCATGCTT	AGGATCCAGGCAGTTTCCTCT-GGAAGG	334			x			x	
Control	MBL exon 4	GAGTTTCACCCACTTTTTCACA	GCCTGAGTGATATGACCCTTC	421							

NOTE. LYPB/LYQC heterozygotes give a weak signal in reaction 1; LYPB/HYPD heterozygotes give a weak signal in reaction 5; reactions 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, and 11 and 12 are complementary. w, weak signal; x, presence of specific PCR product.

infections during early childhood, especially during the first 6–18 months of life [17], and in patients with a concomitant immunodeficiency [18–21]. In addition, several studies have suggested that MBL variant alleles may be weakly associated with autoimmune conditions, such as systemic lupus erythematosus (for a comprehensive meta-analysis, see [22]) and progression of rheumatoid arthritis [23]. However, the importance of MBL as a susceptibility and modifying factor, for the development of sepsis, is unknown.

In the present study, we have investigated, in patients admitted to an academic, multidisciplinary ICU, (1) whether MBL variant alleles confer increased risk of sepsis, severe sepsis, and septic shock in patients with SIRS and (2) whether MBL variant alleles are associated with fatal outcome.

Table 2. Clinical diagnoses at admission to the intensive care unit and positive culture isolates, in 272 consecutive patients with systemic inflammatory response syndrome.

Diagnoses	Nonoperative medical diagnoses	Positive isolates	Postoperative surgical diagnoses	Positive isolates
A. 4 Congestive heart failure	6 (3.4)	0		
B. 15 Pneumonia	69 (38.8)	53 (76.8)		
B. 16 COPD acute deterioration	27 (15.2)	8 (29.6)		
Epiglottitis	7 (3.9)	2 (28.6)		
L. 55 Respiratory infection			39 (41.5)	19 (48.7)
C. 21 Hepatic failure	3 (1.7)	2 (66.7)		
C. 24 G-I inflammatory disease	4 (2.2)	3 (75.0)		
M.59 G-I perforation/rupture/peritonitis			29 (30.9)	7 (24.1)
D. 30 Stroke	3 (1.7)	2 (66.7)		
D. 31 CNS infection	7 (3.9)	4 (57.1)		
D. 33 Neuromuscular	3 (1.7)	3 (100.0)		
D. 34 Seizure (status)	5 (2.8)	4 (80.0)		
E. 37 Urosepsis	12 (6.7)	11 (91.7)		
F. 39 Trauma	6 (3.4)	1 (16.7)		
O.74 Trauma			20 (21.3)	5 (25.0)
G. 41 Ketoacidosis	8 (4.5)	5 (62.5)		
G. 42 Major intoxication	13 (7.3)	5 (38.5)		
Other	5 (2.8)	2 (40.0)	6 (6.4)	0
Total	178 (100.0)	105 (60.0)	94 (100.0)	31 (33.0)

NOTE. Data are no. (%) of patients. Percentages of positive isolates are percentage of number from the column to the left. Nonoperative medical (no surgery within last 7 days) and postoperative surgical (surgery within last 7 days) diagnoses at admittance, according to the APACHE III diagnosis list for critically ill hospitalized adults [30]. Letters and numbers in the first column refer to the APACHE III diagnosis list. CNS, central nervous system; COPD, chronic obstructive pulmonary disease; G-I, gastrointestinal.

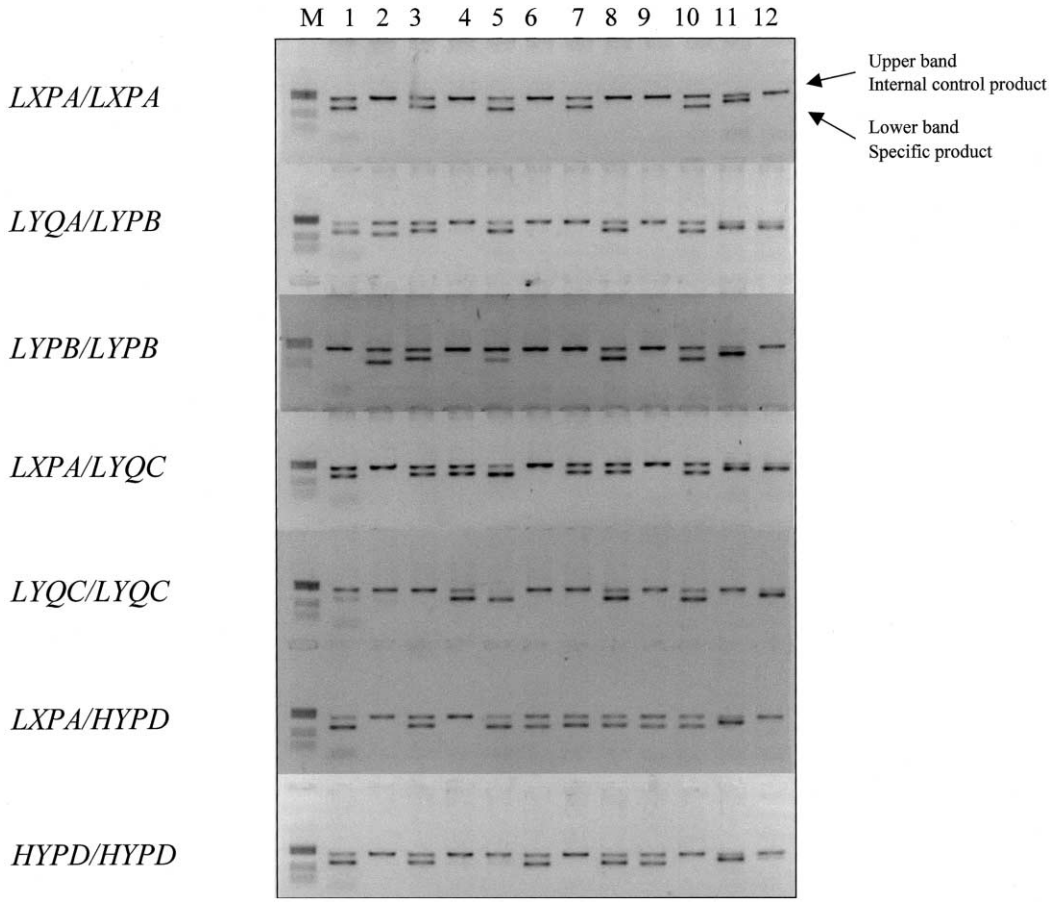

Figure 1. Mannose-binding lectin (MBL) genotyping pattern. MBL genotyping by polymerase chain reaction (PCR) sequence-specific priming. The nos. 1–12 indicate the PCRs listed in table 1. For a detailed description, see Patients and Methods. M, molecular weight marker pBR327/HaeIII.

PATIENTS AND METHODS

From February 1998 to July 1999, all patients >17 years old (range, 18–88 years; 132 women and 140 men) admitted to the academic, multidisciplinary ICU at Glostrup University Hospital, in Copenhagen, Denmark, who met the criteria for SIRS, as outlined by Bone et al. [24], were included in the study. The protocol was approved by the local ethics committee, in the County of Copenhagen. Informed consent was obtained from the patients or from their relatives. The criteria for SIRS and sepsis diagnosis used in this study are outlined in the appendix. Information about death during follow-up, during February 2002, was obtained from the Danish Central Office of Civil Registration. A total of 190 blood donors and 60 members of the hospital staff served as healthy control subjects [16].

Simplified acute physiology score II (SAPS II) and mortality prediction. SAPS II is based on a large international sample of medical and surgical patients and provides an estimate of the risk of fatal outcome, without having to specify a diagnostic category [25]. The "worst values" (specifically outlined in the SAPS II scoring method) within the first 24 h after admission to the ICU were recorded.

Detection of concentrations and genotypes of MBL protein. Concentrations of MBL in serum were measured in a double-enzyme immunoassay, as described elsewhere [26]. The assay preferentially detects fully oligomerized MBL. MBL single-nucleotide polymorphisms (SNPs)—in the form of the structural variants named *B* (codon 54), *C* (codon 57), and *D* (codon 52), as well as the regulatory variants named *H/L* (−550), *X/Y* (−221), and *P/Q* (+4)—were typed by polymerase chain reaction (PCR) using sequence-specific priming, which includes the 12 reactions listed in table 1. We included a PCR accounting for exon 4 of the *mbl2* gene as an internal positive control. The PCR analysis was performed essentially as described elsewhere [27], except that the concentration of dNTPs was reduced to 0.7 m*M*, and the PCR products were analyzed by a 2% agarose gel electrophoresis. Figure 1 shows the 7 patterns necessary to account for all combinations of each of the 6 complementary reactions. The typing system was validated by automated sequencing (ABI 3100 platform) of PCR products from the 7

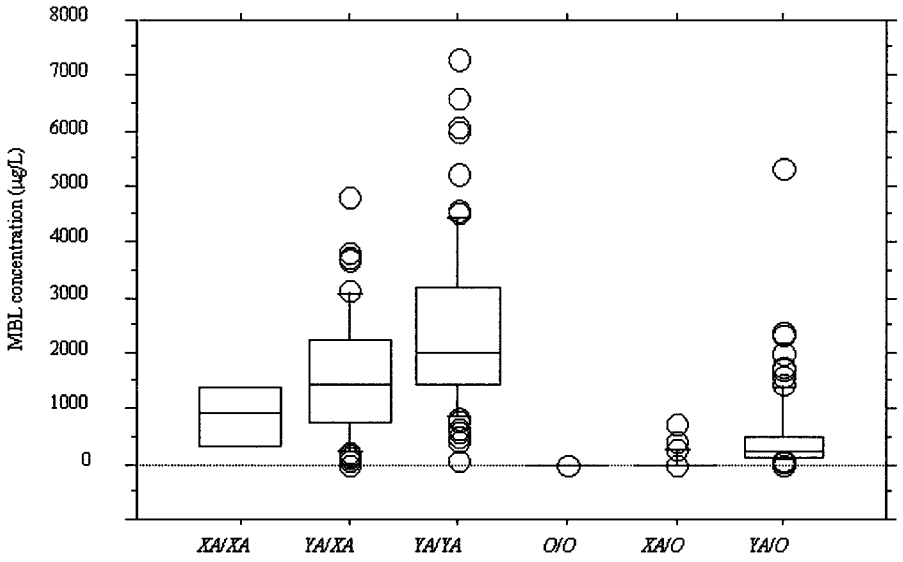

Figure 2. Relationship between concentrations of mannose-binding lectin (MBL) serum and MBL genotypes. Concentrations of oligomerized MBL in serum from 272 patients with systemic inflammatory response syndrome are shown in relation to MBL structural alleles *(0),* as well as the MBL promoter alleles at position −221 *(X/Y).* Ranges, quartiles, and medians are indicated. The detection limit of the assay was 20 μg/L.

control samples accounting for all the polymorphic positions and, in addition, by comparing the typing system with the typing techniques used elsewhere [16]. Although the typing was performed as SNP typing, the results were combined in haplotypes, on the basis of strong linkage disequilibrium between the SNPs, which results in the 7 known haplotypes: 4 functional haplotypes *(LXPA, LYPA, LYQA,* and *HYPA;* the normal allele is designated "*A*") and 3 defective haplotypes *(LYPB, LYQC,* and *HYPD)* [16]. All 3 structural variant alleles *(B, C,* and *D)* have a considerable effect on MBL concentrations, and, to avoid small groups, the 3 alleles were grouped in 1 category, called "allele" *(O),* for statistical analyses. Likewise, for statistical analyses, we only included the *X/Y* promoter variation at position −221. The *X* variant is always found on a functional haplotype *(LXPA)* and has been shown to have a down-regulating effect on MBL expression [16,28]. Thus, the following 6 MBL genotypes/haplotypes were defined: the *A/A* group: 2 normal structural alleles with high-expression promoter activity at position −221 *(YA/YA),* 1 high-expression promoter and 1 low-expression promoter *(YA/XA),* or 2 low-expression promoters *(XA/XA);* the *A/0* group: 1 variant structural allele (i.e., defective allele) and 1 normal structural allele, regulated by a high-expression promoter *(YA/O)* or a low-expression promoter *(XA/O);* and the *O/O* group: 2 defective structural alleles.

Statistical analyses. Contingency table analyses and trend analyses were used to compare frequencies. Kruskal-Wallis or Mann-Whitney tests were used to compare continuous data. When appropriate, logistic-regression analyses were used to investigate for possible confounders. The SAPS II scores were converted into a prediction of mortality, as described elsewhere [25]. To create a current risk of fatal outcome for the present data set, the SAPS II scores were calibrated to the actual mortality, by use of receiver operating characteristic curve analysis and goodness-of-fit tests [29].

RESULTS

Of the 272 patients with SIRS enrolled in the present study, 197 (72.4%) met the criteria for sepsis, either at admission or during the first 24 h in the ICU. Nonoperative (medical) and postoperative (surgical) diagnoses at admission, according to the APACHE III diagnosis list for critically ill, hospitalized adults [30], are outlined in table 2. The distribution of the concentrations of MBL serum was closely associated with the different MBL genotypes (figure 2) ($P < .001$). The frequency of MBL genotypes did not deviate significantly between patients with SIRS and healthy control subjects (table 3). At admission to the ICU, 133 (49%) patients had an infectious, related diagnosis given by the referring physician. In this group of patients, MBL variant alleles were significantly increased, compared with those in patients with a noninfectious, related diagnosis at admission (χ^2 test [2 *df*]; $P < .014$).

Of the 197 patients with sepsis, 171 (86.8%) met the criteria for severe sepsis (table 4). Furthermore, 70 (40.9%) of these patients met the criteria for septic shock. Stratification of the patients, according to MBL genotypes, revealed that a highly significant proportion of the patients with sepsis carried MBL

Table 3. Mannose-binding lectin (MBL) genotypes in healthy control subjects versus all patients with systemic inflammatory response syndrome admitted to an intensive care unit.

Allele, genotype	Control subjects	All patients
Structural alleles[a]		
Sum *A/A*	157 (62.8)	151 (55.5)
A/B	48 (19.2)	59 (21.7)
A/C	13 (5.2)	8 (2.9)
A/D	25 (10.0)	40 (14.7)
Sum *A/O*	86 (34.4)	107 (39.3)
B/B	3 (1.2)	5 (1.8)
B/D	3 (1.2)	7 (2.6)
D/D	1 (0.4)	2 (0.7)
Sum *O/O*	7 (2.8)	14 (5.1)
Total	250 (100.0)	272 (100.0)
Promoter alleles included[b]		
YA/YA	72 (28.8)	83 (30.5)
YA/XA	73 (29.2)	65 (23.9)
XA/XA	12 (4.8)	3 (1.1)
Sum*A/A*	157 (62.8)	151 (55.5)
YA/O	53 (21.2)	82 (30.1)
XA/O	33 (13.2)	25 (9.2)
Sum *A/O*	86 (34.4)	107 (39.3)
Sum *O/O*	7 (2.8)	14 (5.1)
Total	250 (100.0)	272 (100.0)

NOTE. Data are no. (%) of subjects. Control subjects versus patients *(A/A* vs. *A/O* plus *O/O).Y* and *X* indicate base exchanges at codon −221, which profoundly influence the expression of MBL. *X* is present only on *A* chromosomes. *A,* normal structural allele; *O,* variant alleles (*B,* codon 54; *C,* codon 57; and *D,* codon 52).

[a] χ^2 test for linear trend, 3.67 ($P = .06$).
[b] χ^2 test for linear trend, 0.51 ($P = .47$).

variant alleles, compared with the patients without sepsis (table 4) ($P < .001$). Further analyses showed that patients carrying MBL variant alleles also had a high risk of developing severe sepsis and septic shock ($P < .001$).

When the promoter alleles were taken into account, there was a striking linear trend in susceptibility to sepsis, severe sepsis, and development of septic shock, from the highest-expressing MBL genotypes to genotypes encoding MBL deficiency, compared with that for patients with SIRS without sepsis (table 4) ($P < .001$). Moreover, an independent and significant association between the presence of MBL variant alleles *(A/A* vs. *A/O* plus *O/O)* and sepsis ($n = 26$) was observed when the patients with severe sepsis and septic shock were excluded from the analysis (χ^2 test, 4.77 [1 *df*]; $P = .028$) and also when patients with septic shock were excluded from the analysis of the severe-sepsis group ($n = 101$) (χ^2 test, 5.25 [1 *df*]; $P = .022$). Likewise, the association was also seen when comparing patients with SIRS with sepsis with healthy control subjects (χ^2 test for linear trend, 10.8; $P = .001$) (tables 3 and 4).

A gradual decrease in concentrations of MBL serum was seen with increased severity of sepsis (SIRS, sepsis, severe sepsis, and shock) ($P = .0032$, Kruskal-Wallis), which was in full accordance with the data on genotype. Age was a significant risk factor for developing sepsis ($P = .03$, logistic regression). However, when a logistic-regression model was used, the risk of developing sepsis in patients carrying MBL variant alleles was independent of age ($P < .001$).

Specimens obtained immediately before or at admission to the ICU were culture positive for 50% of the patients (table 5). The microbiological spectrum was wide, and no infectious agent was predominant. However, a significantly increased proportion of the patients carrying MBL variant alleles were culture positive in a gene dose-dependent manner ($P = .012$). It is noteworthy that MBL deficiency was associated with both gram-negative and gram-positive bacteria, but an association with fungal infections was less certain.

In total, 83 (30.5%) of the patients died while admitted to hospital (table 6). Among these patients, 25.8% of those with the normal genotype, 34.6% with the heterozygous genotype, and 50.0% with the homozygous defective genotype died during the in-hospital period (χ^2 test for linear trend, 4.7; $P = .030$) (table 6). Inclusion of the MBL promoter alleles suggested that those patients with the highest levels of MBL *(YA/YA)* were the most protected against fatal outcome (χ^2 test for linear trend, 4.7; $P = .030$) (table 6). It should be noted that an increased risk of fatal outcome, for patients carrying MBL variant alleles, was present in both the sepsis group and the nonsepsis group, but neither was significant at the 5% level. Also, a reduced concentration of MBL serum was observed in the nonsurvivor group, compared with that in the survivor group (mean ± SD, 997 ± 1225 and 1398 ± 1456 μg/L, respectively; $P = .020$).

No significant differences in the parameters underlying the SAPS II score, the crude SAPS II score, and the predicted standardized mortality rate (observed mortality divided by predicted mortality), on the basis of the SAPS II score, were observed when stratified according to MBL genotypes (table 7) ($P > .05$), nor was there any significant association between increasing numbers of SIRS criteria (2 to 4) and the MBL genotypes. Separating age score from SAPS II score revealed that, when tested by logistic regression, the association between the MBL genotypes and fatal outcome was independent of both the age score and the acute physiology score (APS) ($P = .025$, for MBL genotypes; $P < .001$, for age and APS).

During the follow-up period, an additional 68 patients died (mean follow-up time, 30.3 months). During this period, no significant overall association was seen between MBL variant alleles and fatal outcome (χ^2 test for trend, 0.22; $P = .64$).

DISCUSSION

Numerous factors are involved in controlling and limiting localized infections. In general, the septic response occurs when

Table 4. Mannose-binding lectin genotypes, structural alleles, comparisons of patients without sepsis and those with sepsis, and severe sepsis and septic shock, in patients with systemic inflammatory response syndrome.

Allele, genotype	No. (%) of patients				Risk ratio (95% CI)[a]		
	Without sepsis	With sepsis	With severe sepsis	With septic shock	Sepsis	Severe sepsis	Septic shock
Structural alleles							
Sum *A/A*	55 (73.3)	96 (48.7)	83 (48.5)	31 (44.3)	1	1	1
A/B	9 (12.0)	50 (25.4)	45 (26.3)	14 (20.0)			
A/C	2 (2.7)	6 (3.1)	5 (2.9)	3 (4.3)			
A/D	9 (12.0)	31 (15.7)	26 (15.2)	13 (18.6)			
Sum *A/O*	20 (26.7)	87 (44.2)	76 (44.4)	30 (42.9)	1.28 (1.1–1.49)	1.32 (1.11–1.56)	1.66 (1.16–2.39)
B/B	0	5 (2.5)	4 (2.3)	3 (4.3)			
B/D	0	7 (3.6)	6 (3.5)	4 (5.7)			
D/D	0	2 (1.0)	2 (1.2)	2 (2.9)			
Sum *O/O*	0	14 (7.1)	12 (7.0)	9 (12.9)	1.57 (1.39–1.77)	1.66 (1.45–1.9)	2.77 (2.09–3.68)
Total	75 (100.0)	197 (100.0)	171 (100.0)	70 (100.0)			
Promoter alleles included							
YA/YA	31 (41.3)	52 (26.4)	45 (26.3)	14 (20.0)	1	1	1
YA/XA	24 (32.6)	41 (20.8)	36 (21.1)	17 (24.3)	1.01 (0.78–1.29)	1.01 (0.77–1.34)	1.33 (0.76–2.35)
XA/XA	0	3 (1.5)	2 (1.2)	0	1.60 (1.35–1.88)	1.69 (1.40–2.04)	NA
YA/O	17 (22.7)	65 (33.0)	55 (32.2)	21 (30.0)	1.27 (1.04–1.54)	1.29 (1.03–1.62)	1.78 (1.06–2.99)
XA/O	3 (4.0)	22 (11.2)	21 (12.3)	9 (12.9)	1.40 (1.13–1.75)	1.48 (1.16–1.88)	2.41 (1.40–4.15)
O/O	0	14 (7.1)	12 (7.0)	9 (12.9)	1.60 (1.35–1.88)	1.69 (1.40–2.04)	3.21 (2.08–4.96)

NOTE. CI, confidence interval.

[a] *A/A* vs. *A/O* plus *O/O*. χ^2 for linear trend, <15.1 ($P < .001$).

[b] χ^2 for linear trend, <15.8 ($P < .001$).

immune defenses fail to contain an invading microbe. Deficiencies in nonadaptive, innate host factors have been suggested to be of particular importance [3], but, so far, the epidemiological proof of such a notion has been limited. The present study has indicated that functional MBL is important for avoiding the development of sepsis and septic shock in critically ill patients. We have found that patients with SIRS have a high risk of developing sepsis, provided that they carry variant alleles in the *mbl2* gene, which decrease the level of functional MBL in the blood. Although not as prominent, the same difference was observed when we compared patients with sepsis and healthy control subjects. Thus, MBL seems to be involved in controlling systemic dissemination of different infectious agents in patients with acute medical and surgical stress. This observation is in agreement with the finding that a significantly increased proportion of patients carrying MBL variant alleles also had a positive culture for microbial species.

In general, MBL exerts its largest effect during the vulnerable window of infancy, especially during the first 6–18 months of life [17]. Nevertheless, MBL deficiency has been associated with a number of infections, particularly in patients with concomitant immunodeficiencies [18–21]. It is conceivable that the initial SIRS insult creates a precondition rendering the patient partly immunocompromised. This increases the patient's susceptibility to infection, thereby exposing the clinical MBL phenotype. The necessity that an accompanying condition should be present before MBL deficiency becomes clinically important has recently been indicated in relation to pneumococcal pneumonia. In unselected patients, a variable association is seen [31, 32], whereas, in selected patients with a concomitant disorder, a clear association is seen [19, 22].

The finding that the frequency of MBL variant alleles increases with severity of sepsis (severe sepsis and septic shock) indicates that the lack of buffering capacity in MBL, toward initial microbial replication, not only is associated with susceptibility to infection, but also may allow the activation of host mechanisms central to the pathophysiology of the sepsis syndrome. Consistent with this view are several in vitro findings indicating that MBL may suppress the release of proinflammatory cytokines [33, 34]. Thus, MBL may both play a direct antimicrobial role and have a modulating effect on the inflammatory response. In a recent study, MBL has been shown to function as a scavenger molecule toward cells undergoing processes of apoptosis [35] and necrosis [36]. Dysfunctional handling of dying host cells could be of importance for the pathophysiology of sepsis, and lack of functional MBL could theoretically, by such a mechanism, become clinically relevant for the progression of the sepsis syndrome.

During hospital stay, an increased risk of fatal outcome was observed in the patients carrying MBL variant alleles. By contrast, no such association was observed during the 30 months of follow-up, indicating that the increased risk of fatal outcome

Table 5. Nos. of patients and microbial species diagnosed in cultures obtained at admission (day −3 to +1) to intensive care unit, in 272 consecutive patients with systemic inflammatory response syndrome.

Patient or microorganism	Total	*A/A*	*A/O*	*O/O*
Patient				
Sum positive	136 (50.0)	68 (45.0)[a]	56 (52.3)[a]	12 (85.7)[a]
Sum negative or unknown	136 (50.0)	83 (55.0)[a]	51 (47.7)[a]	2 (14.3)[a]
Total	272	151	107	14
Microorganisms				
Gram positive	65 (47.7)	34 (50.0)	25 (44.6)	6 (50.0)
Staphylococcus aureus	21 (32.3)	11 (32.4)	9 (36.0)	1 (16.7)
Streptococcus pneumoniae	17 (26.2)	8 (23.5)	6 (24.0)	3 (50.0)
Staphylococcus albus	15 (23.0)	10 (29.4)	5 (20.0)	0
Streptococcus faecalis	6 (9.2)	1 (2.9)	4 (16.0)	1 (16.7)
Streptococcus pyogenes	2 (3.1)	2 (5.9)	0	0
Other gram positive	4 (6.2)	2 (5.9)	1 (4.0)	1 (16.7)
Gram negative	57 (41.9)	27 (39.7)	25 (44.6)	5 (41.7)
Escherichia coli	24 (42.1)	10 (37.0)	10 (40.0)	4 (80.0)
Haemophilus influenzae	13 (22.8)	6 (22.2)	7 (28.0)	0
Klebsiella pneumoniae	5 (8.8)	1 (3.7)	4 (16.0)	0
Pseudomonas aeruginosae	3 (5.3)	3 (11.1)	0	0
Serratia marcescens	3 (5.3)	3 (11.1)	0	0
Branhamella catarrhalis	3 (5.3)	2 (7.4)	1 (4.0)	0
Other gram negative	6 (10.6)	2 (7.4)	3 (12.0)	1 (20.0)
Fungi				
Candida albicans	14 (10.3)	7 (10.3)	6 (10.7)	1 (8.3)

NOTE. A total of 173 cultures (40 from blood, 97 from trachea, 26 from urine, and 10 from other sources) obtained from 136 patients were found to be culture positive. A species appearing in >1 culture/patient was counted only once. *A,* normal structural allele; *O,* common designation for variant alleles (*B*, codon 54; *C*, codon 57; and *D*, codon 52)

[a] χ^2 test for linear trend, 6.02 ($P = .012$).

was directly related to the actual pathological incidence. However, the close association between MBL deficiency and the development of sepsis, which was higher than the association between MBL deficiency and fatal outcome, suggests that MBL may influence processes related to initial steps in the disease process. Consistent with this view is the lack of association both between MBL variant alleles and the SAPS II score and between MBL variant alleles and an increasing number of SIRS criteria. Both increased SAPS II score and increasing number of criteria are independently associated with the risk of fatal outcome [25]. In this regard, it is interesting that excessive activation of complement, which takes place in SIRS and sepsis, has been shown to be associated with fatal outcome [37]. Moreover, genetic complement deficiency and complement depletion have been shown to be beneficial in animal models of complement-dependent inflammation [38]. Thus, complement and, probably, MBL may play different roles during the course of the sepsis syndrome, which may explain why it is difficult to classify patients with SIRS with aberrations in their MBL genetic profile, in relation to prognosis. The possibility of confounding factors—for example, treatment given and duration of illness before admission to the ICU—that have not been revealed by us should also be considered.

We only included white patients of Danish origin. However, the ethnic-specific genetic constitution, as well as nongenetic factors, will vary in different studies. Thus, to establish the full effect that MBL deficiency has on the sepsis syndrome, these findings need to be replicated in other populations, as well as in settings with different microbial regimens and clinical surveillance regimes.

Table 6. Manose-binding lectin genotypes in patients with systemic inflammatory response syndrome (comparison between survivors and nonsurvivors during stay at hospital).

Allele, genotype	Survivors	Nonsurvivors	Risk ratio (95% CI)
Structural alleles			
Sum*A/A*	112 (59.3)	39 (47.0)	1
A/B	38 (20.1)	21 (25.3)	
A/C	5 (2.6)	3 (3.6)	
A/D	27 (14.3)	13 (157)	
Sum*A/O*	70 (37.0)	37 (44.6)	1.34 (0.92–1.95)
B/B	4 (2.1)	1 (1.2)	
B/D	1 (0.5)	6 (7.2)	
D/D	2 (1.1)	0 (0.0)	
Sum*O/O*	7 (3.7)	7 (8.4)	1.94 (1.07-3.49)
Total	189 (100.0)	83 (100.0)	
Promoter alleles included			
YA/YA	65 (34.4)	18 (21.7)	1
YA/XA	44 (23.3)	21 (25.3)	1.49 (0.87–2.55)
XA/XA	3 (1.6)	0	NA
YA/O	53 (28.0)	29 (34.9)	1.63 (1.0–2.70)
XA/O	17 (9.0)	8 (9.6)	1.48 (0.7–2.98)
O/O	7 (3.7)	7 (8.4)	2.31 (1.19–4.48)
Total	189 (100.0)	83 (100.0)	

NOTE. Data are no. (%) of subjects, unless otherwise noted. χ^2 test for linear trend, 4.7 ($P = .030$). CI, confidence interval.

Table 7. Simplified Acute Physiology Score (SAPS II) parameters and observed and predicted mortality in 272 patients with systemic inflammatory response syndrome (SIRS), classified by mannose-binding lectin structural variant alleles.

Variable	Total (n = 272)	*A/A* (n = 151)	*A/O* (n = 107)	*O/O* (n = 14)
Age, mean ± SD, years	60.2 ± 16.5	60.1 ± 16.7	60.7 ± 16.3	55.5 ± 17.8
Type of admission				
Acute surgery	86 (31.6)	51 (33.8)	33 (30.8)	2 (14.3)
Elective surgery	8 (2.9)	5 (3.3)	3 (2.8)	0
Medical[a]	178 (65.4)	95 (62.9)	71 (66.4)	12 (85.7)
Chronic disease[b]				
AIDS	0	0	0	0
Metastatic cancer	1	1	0	0
Hematological malignancy	3	3	0	0
SAPS II				
SAPS II score, mean ± SD	37.7 ± 15.4	36.8 ± 15.1	38.4 ± 16.3	42.1 ± 10.6
Age, mean ± SD, points	10.4 ± 5.4	10.4 ± 5.2	10.4 ± 5.7	10.1 ± 5.7
Acute physiology, mean ± SD, points[c]	27.2 ± 13.3	26.4 ± 12.7	28.0 ± 14.4	30.4 ± 11.2
Mortality				
Mortality observed	83 (30.5)	39 (25.8)	37 (34.6)	7 (50.0)
Mortality predicted[d]	83.0 (30.5)	44.6 (29.5)	33.4 (31.2)	5.0 (35.7)
SMR, mean	1.00	0.87	1.11	1.40
No. of SIRS criteria				
2	71 (26.1)	43 (28.5)	27 (25.2)	1 (7.1)
3	91 (33.5)	51 (33.8)	36 (33.6)	4 (28.6)
4	110 (40.4)	57 (37.7)	44 (41.1)	9 (64.3)

NOTE. Data are no. (%) of subjects, unless otherwise noted. *A*, normal structural allele; *O*, common designation of the variant alleles (*B*, codon 54; *C*, codon 57; and *D*, codon 52); SMR, standardized mortality rate (mortality observed/mortality predicted).

[a] Without surgery within last 7 days.

[b] Chronic disease is based on available information on day 1.

[c] Acute physiology score, combined score for heart rate, systolic blood pressure, temperature, white blood cell count, PaO_2/FiO_2 ratio, urinary nitrogen, bilirubin, natrium, potassium, bicarbonate, diuresis, and Glasgow Coma Score.

[d] Predicted mortality after first-level customization (logit,-3,8084+(0,0741*SAPS II). Continuous data was analyzed by Kruskal-Wallis test and categorical data by χ^2 test. Between the different MBL genotypes, none of the parameters deviated significantly ($P > .05$).

In conclusion, the present study has shown that genetically determined differences in the MBL gene *(mbl2)* explain a significant proportion of the inherited risk of developing sepsis in critically ill patients. MBL variant alleles were also associated with increased risk of fatal outcome. Accordingly, rapid determination of the MBL genotype could be important for identification of patients who are at risk for developing severe sepsis. Moreover, since MBL-substitution therapy is now possible [39], our data raise the prospect that MBL may be used in prophylaxis and treatment of the sepsis syndrome.

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APPENDIX

SUMMARY OF CLASSIFICATION CRITERIA (SIRS, SEPSIS, SEVERE SEPSIS, AND SEPTIC SHOCK)

Systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic shock were defined in accordance with the recommendations of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference [24]. Patients were required to meet ≥2 of the following 4 SIRS criteria: (1) a core temperature of ≥38°C or ≤36°C; (2) a heart rate of >90 beats/min; (3) a respiratory rate of ≥20 breaths/min, a $PaCO_2$ ratio of ≤4.3 kPa (32 mm Hg), or a need for mechanical ventilation; (4) or a white blood cell count of $\geq 12.0 \times 10^9$ cells/L or $\leq 4.0 \times 10^9$ cells/L or a differential count

showing >10% immature neutrophils. Subsequently, the criteria for sepsis was SIRS with a documented infection or a clinically suspected infection—such as postoperative intra-abdominal sepsis in which the intestinal tract either has been perforated or requires partial resection for ischemia, intestinal content appearing intra-abdominally due to a leak from prior gut anastomosis, and radiographic evidence of pneumonia in association with purulent sputum. Infection was documented by a positive culture or convincing Gram stain, obtained no more than 3 days before admission to the ICU. Severe sepsis was defined as sepsis and either hypotension or evidence of hypoperfusion and organ dysfunction, developing within 24 h of enrollment in the study. Patients were required to meet at least 1 of the following criteria to be defined as having organ dysfunction: (1) arterial systolic blood pressure of <90 mm Hg for at least 1 h, despite appropriate fluid resuscitation, or vasopressor therapy to maintain a systolic blood pressure >90 mm Hg; (2) urine output of <0.5 mL/kg for >1 h, despite hydration; (3) PaO_2/FiO_2 ratio of ≤40 kPa (300 mm Hg); (4) an acute alteration in mental status (Glasgow Coma Score <14); (5) unexplained metabolic acidosis, with pH ≤7.30 or base deficit of ≥5.0 m*M*, in association with an increased level of plasma lactate of ≥1.6 m*M*; or (6) hepatobiliary dysfunction with serum bilirubin of >34 μM and no evidence of preexisting hepatobiliary disease.

Septic shock was defined as sepsis with hypotension in combination with 1 of the other criteria for acute organ dysfunction. Patients were excluded from participation if any of the following conditions were present: neutrophil count of $<1.0 \times 10^9$ cells/L before the onset of sepsis, infections associated with burns, documented or suspected recent acute myocardial infarction, or lack of commitment to full life-support measures by the primary physician.

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