The Influence of Mannose Binding Lectin Polymorphisms on Disease Outcome in Early Polyarthritis

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ABSTRACT. ***Objective.*** To determine whether variant alleles of the mannose binding lectin (MBL) gene causing low serum concentrations of MBL are associated with increased susceptibility to rheumatoid arthritis (RA) and erosive outcome in an inception cohort of patients with early polyarthritis.

Methods. MBL and HLA-DRB1 alleles were determined by polymerase chain reaction in 68 Danish patients with incident early polyarthritis observed for one year. The associations between MBL and specific HLA-DRB1 genotypes and disease outcomes were analyzed.

Results. Among the patients with early polyarthritis 7.4% (5/68) and 41.2% (28/68) were homozygous and heterozygous for MBL variant alleles, compared with 2.8% (7/250) and 34.4% (86/250) of healthy controls ($p = 0.09$), while the corresponding figures in the patients with RA were 10% (5/50) and 42% (21/50) ($p = 0.03$), and in the patients with erosive RA 18.8% (3/16) and 35.3% (6/16), respectively ($p = 0.004$). Patients with early polyarthritis homozygous for MBL variant alleles had an increased risk of having erosive RA at inclusion by a factor of 4.7 ($p = 0.02$) and after one year by a factor of 3.6 ($p = 0.04$). MBL deficiency was associated with increased levels of C-reactive protein (CRP) and IgM rheumatoid factor (RF) at inclusion ($p < 0.05$). HLA-DRB1 alleles were not found to be associated with disease outcome.

Conclusion. MBL variant alleles appear to be weak susceptibility markers for RA, and patients with early polyarthritis and homozygous for MBL structural variant alleles have a higher risk of developing early erosive RA. These findings, together with the positive association between MBL variant alleles and the increased serum levels of IgM RF and CRP, point at the MBL gene as a relevant locus in the pathophysiology of RA. (J Rheumatol 2001;28:935–42)

Key Indexing Terms:

RHEUMATOID ARTHRITIS	GENETICS	INNATE IMMUNITY
MANNOSE BINDING LECTIN	HLA-DR ANTIGENS	DISEASE OUTCOME

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial inflammation, breakdown of articular cartilage, and destruction of subchondral bone. The pathogenesis of RA is unknown, but RA is generally considered to be an autoimmune disease in which environmental, hormonal, and genetic factors may initiate or modulate the inflammatory response. Several putative autoantigens are described as targeted by B cells and/or T cells in RA[1]. One of the best known autoantigens in RA, which is ubiquitously expressed, is the class G immunoglobulin (IgG), which may become the target of other antibodies, i.e., rheumatoid factors (RF). Immune complexes of RF may generate synovial inflammation by means of complement activation or cross linking of Fcγ-receptors on synovial macrophages[2]. The presence of RF is not only of pathogenetic significance, but has also a negative influence on the outcome of RA[3,4].

The potential immunogenetic role of HLA-DRB1 alleles in the pathogenesis and outcome of RA has been extensively studied, but is still controversial[3,4]. A recent large British study of an inception cohort of patients with early inflammatory arthritis did not reveal any associations between HLA-DRB1 alleles and disease persistency, and only showed a slightly increased risk of erosive outcome in the presence of a shared epitope[3].

Mannose binding lectin (MBL) is a liver derived serum protein involved in innate immune defense. The ligands for

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MBL are high mannose and *N*-acetylglucosamine oligosaccharides, expressed by a wide range of microorganisms[5]. Serum MBL utilize novel enzymes to activate the complement systems (MASP) and can directly opsonize pathogens and enhance the activity of phagocytes via receptors it shares with complement C1q and lung surfactant protein A[6–8]. The serum MBL concentrations exhibit large interindividual variations because of variant alleles in the structural moiety of the *mbl2* gene located in humans on chromosome 10. An MBL pseudo gene (mbl1) is situated at the same chromosome[9]. In exon 1 of the *mbl2* gene, 3 different point mutations have been described at codon 54 (*B* allele), codon 57 (*C* allele), and codon 52 (*D* allele)[10-13]. The common designation for these variant alleles is *O* whereas the normal allele has been named *A*. Each of the 3 variants independently reduces the amount of functional MBL subunits. Moreover, several point mutations of the promoter region of the *MBL* gene affect the serum MBL level[14,15]. Particularly, a polymorphism in codon –221 (*X* type) downregulates serum MBL concentration[14,15]. MBL variant alleles causing low serum levels of functional MBL have been shown to be associated with increased risk of infections during childhood[16,17], as well as in adults[18,19]. MBL may also influence the expression of diseases[19-21]. Moreover, MBL has been shown to modulate the phenotypic expression of RA because low serum levels of MBL have been shown to be associated with young age at onset of RA and poor disease outcome[22]. Similar findings have been made in RA patients with variant alleles[23,24].

In our study of patients with early polyarthritis, we assessed the influence of MBL and HLA-DRB1 alleles on disease outcome. Adverse clinical outcomes were defined as having RA and appearance of erosive changes.

MATERIALS AND METHODS

Patients and controls. Patients had symmetrically swollen or tender 2nd or 3rd metacarpophalangeal or proximal interphalangeal joints and a symptom duration of less than 2 years. All patients were part of an extensive study of consecutive patients with early polyarthritis as reported in other substudies[25,26]. All had given informed consent to participate in the study. The date of onset was defined as the date when the patient recalled having joint pain or swelling of the above-mentioned joints for the first time. Two hundred fifty unrelated healthy white Danes (190 blood donors and 60 members of the hospital staff) served as controls for the genetic MBL analyses. Another group of 192 controls were typed for HLA-DRB1 alleles.

Clinical and laboratory assessment. For each patient a full history and examination was performed at first visit. Repeated assessments of clinical activity, functional impairment, and whether the patients fulfilled 1987 American College of Rheumatology (ACR) list or tree classification criteria for RA[27] were performed monthly for 2 years. In this study we report data after the first year of observation. These assessments included the measures listed in the ACR preliminary improvement criteria for RA[28], i.e., number of swollen and tender joints (out of 28), Health Assessment Questionnaire (HAQ) score[29], global evaluation by the patient and the doctor on a 10 cm visual analog scale (0 best possible, 10 worst possible). C-reactive protein (CRP) and erythrocyte sedimentation rate (not reported here) were measured at every visit. IgM RF was measured at inclusion and at month 3, 6, and 12 as long as the patient was seronegative. The detection level of the CRP assay was 95 mg/dl and the upper normal range of the IgM RF test was 17 units. Radiographs of hands and wrists were planned at month 0, 3, 6, and 12. All radiographs were evaluated by the same experienced radiologist in terms of bone erosions and Larsen score calculation (not reported here).

Treatment. Patients were allowed to use nonsteroidal antiinflammatory drugs and simple analgesics. The main treatment principle aimed at maximal inhibition of inflammatory activity by using intramuscular or intraarticular corticosteroids and disease modifying antirheumatic drugs (DMARD) during periods of inflammatory activity. Sulfasalazine or methotrexate monotherapy were first choice treatments.

Study outcomes. Two main adverse clinical outcomes were defined: (1) fulfillment of either tree or list classification criteria for RA[27] and (2) presence of erosive RA changes evaluated by hand radiographs. All patients were to be followed for at least 2 years. In the present study, the cohort was evaluated at inclusion and after the first year of followup.

Detection of MBL protein concentrations and genotypes. MBL concentrations in serum were measured in a double-enzyme immunoassay as described[30]. Genomic DNA was isolated from EDTA blood cells and stored at –20°C[31]. DNA was amplified by general polymerase chain reaction (PCR) and the MBL alleles were detected as described[13]. MBL genotyping was expanded with determination of a downregulating MBL promoter allele *X* at codon –221 versus a normal expressing allele (*Y*)[14,15]. The *X* allele is only present on a functional haplotype[13]. The following 6 MBL genotypes could thus be identified. The *A/A* group: 2 normal structural alleles with high-expression promoter activity in position –221 (*YA/YA*), or 1 high-expression promoter and 1 low-expression promoter (*YA/XA*), or 2 low-expression promoters (*XA/XA*). The *A/O* group: 1 variant structural allele and 1 normal structural allele combined with a high-expression promoter (*YA/O*) or a low-expression promoter (*XA/O*). The *O/O* group: 2 structurally defective alleles.

Because *A/O* individuals carrying the low-expressing *X* promoter allele have very low serum MBL levels (Figure 1), we pooled these patients with those homozygous for 2 defective structural alleles (*O/O*) into an *XA/O + O/O* group and the rest of the patients into a *YA/O + A/A* group.

Determination of HLA-DRB1 alleles. Genomic "low resolution" HLA-DRB1 tissue typing was performed by PCR based sequence-specific oligonucleotide probing as described[12,32].

Statistical analyses. Comparison of nonpaired categorical variables was performed by contingency table analysis and Fisher's exact test. Different strata of continuous variables were described and compared using nonparametric methods. Statistical processing of the study data was performed using the software package Epi Info 6[33].

RESULTS

Demographic and clinical features. Blood samples for DNA extraction were collected in 68 patients included in an early polyarthritis cohort during a 2 year period from June 1996 to March 1998. The patients consisted of 12 men and 56 women with a median age at inclusion of 53 (range 20–83) years (Table 1). The median duration of symptoms prior to inclusion was 3 (range 0–24) months. Of the 68 patients, one patient with RA at inclusion died of pulmonary embolism just before the planned radiographic followup at 6 months, and 7 patients without RA at inclusion and remission of symptoms chose to withdraw from further controls within 4 months of followup.

After one year of followup, 50 patients had RA including 16 patients with erosive disease. DMARD treatment was initiated in 52 patients. Fifty-two patients started DMARD

Table 1. Demographic, basic clinical, and laboratory characteristics of the 68 patients with early polyarthritis by mannose-binding lectin (MBL) genotype.

	All Patients	MBL Genotype *A/A*	*A/O*	*O/O*	MBL Expanded Genotype *YA/O + A/A*	*XA/O + O/O*	p*	p†
Demographic variables								
No. of patients (%)	68 (100)	35 (52)	28 (41)	5 (7)	54 (79)	14 (21)		
No. of males (%)	12 (19)	7 (20)	5 (18)	0	10 (19)	2 (14)	0.58	1.00
Age (yrs) at study entry	53 (20–83)	54 (24–82)	44 (20–83)	53 (36–82)	54 (21–83)	40 (20–82)	0.91	0.07
Symptom duration at entry, mo‡	4 (0–24)	4 (0–24)	4 (0–24)	10 (3–13)	4 (0–24)	9 (3–24)	0.05	0.002
Clinical variables								
Patient pain assessment, mm	35 (0–84)	29 (0–84)	35 (3–67)	40 (15–73)	33 (0–84)	37 (3–73)	0.32	0.69
Patient ass. of disease activity, mm	44 (0–88)	44 (0–88)	44 (3–84)	54 (16–75)	45 (0–88)	43 (3–75)	0.37	0.77
HAQ score	0.9 (0–2.4)	0.9 (0–2.4)	0.9 (0–2.0)	1.0 (0–1.5)	0.9 (0–2.4)	0.7 (0–1.5)	0.81	0.16
No. of tender joints	16 (0–24)	16 (0–24)	16 (0–24)	20 (4–21)	16 (0–24)	17 (0–22)	0.45	0.82
No. of swollen joints	6 (0–18)	6 (0–18)	6 (0–15)	4 (0–15)	6 (0–18)	2 (0–15)	0.77	0.06
Physician global assessment, mm	19 (0–79)	19 (0–79)	20 (0–47)	10 (6–47)	23 (0–79)	11 (0–47)	0.56	0.04
Receiving DMARD treatment (%)	52 (77)	27 (77)	20 (71)	5 (100)	41 (76)	11 (79)	0.33	1.00
Laboratory tests								
CRP, mg/dl	95 (95–1374)	95 (95–543)	95 (1374)	189 (95–1365)	95 (95–1374)	95 (95–1365)	0.02	0.93
IgM rheumatoid factor, units	9 (9–508)	9 (9–189)	11 (9–508)	67 (9–504)	9 (9–508)	56 (9–504)	0.05	0.02

*Comparison of groups *A/A* and *A/O* vs group *O/O* by means of 2-sided Fisher's exact test and Mann-Whitney test.
†Comparison of groups *YA/O+A/A* and *XA/O+O/O* by means of 2-sided Fisher's exact test and Mann-Whitney test.
‡Time span between onset of arthralgia/swelling and study entry.

treatment during the study. DMARD therapy was altered in 31 patients; in 26 patients, sulfasalazine was substituted with methotrexate. The DMARD used were sulfasalazine monotherapy (1), methotrexate monotherapy (35), penicillamine monotherapy (2), hydroxychloroquine monotherapy (1), methotrexate + sulfasalazine + hydroxychloroquine (1), and methotrexate + cyclosporine (1).

MBL and HLA-DRB1 polymorphisms. Thirty-five of the 68 patients with early polyarthritis expressed the normal genotype *A/A* (51.5%), 28 had genotype *A/O* (41.2%), and 5 had *O/O* (7.4%). No deviation from Hardy-Weinberg expectations was seen in either the patient group or the control group. The frequencies of the *A/A*, *A/O*, and *O/O* genotypes differed slightly between patients and controls, without reaching statistical significance (chi-squared = 4.8, 2 df, p = 0.09). This difference in distributions was more pronounced comparing controls with patients with RA (chi-squared = 7.6, 2 df, p = 0.02), and even more compared with patients who had erosive RA (chi-squared = 11.1, 2 df, p = 0.004) (Table 2). The frequency of the *O* allele was 0.20 in controls, 0.28 in the whole patient group, 0.31 in patients who had RA, and 0.38 in patients who had erosive RA. Distribution of MBL downregulating promoter allele *X* was not significantly different in the early polyarthritis patients compared

Table 2. Frequencies of mannose-binding lectin genotypes in 68 Danish patients with early polyarthritis (EPA) and controls. Data for patients with and without rheumatoid arthritis (RA) and erosive RA (eRA) after one year of observation are also shown.

	Controls	All Patients	Patients without RA	Patients with RA	Patients with eRA	OR, All vs Controls*	OR, No RA vs Controls	OR, RA vs Controls†	OR, Erosive RA vs Controls‡
A/A	157 (62.8)	35 (51.5)	11 (61.1)	24 (48.0)	7 (43.8)	1	1	1	1
A/B	48 (19.2)	17 (25.0)	6 (33.3)	11 (22.0)	2 (12.5)				
A/C	13 (5.2)	3 (4.4)	0	3 (6.0)	1 (6.3)				
A/D	25 (10.0)	8 (11.8)	1 (5.6)	7 (14.0)	3 (18.8)				
Sum *A/O*	86 (34.4)	28 (41.2)	7 (38.9)	21 (42.0)	6 (37.5)	1.5 (0.8–2.7)	1.2 (0.4–3.4)	1.6 (0.8–3.2)	1.6 (0.4–5.6)
B/B	3 (1.2)	1 (1.5)	0	1 (2.0)	1 (6.3)				
B/C	0	1 (1.5)	0	1 (2.0)	0				
B/D	3 (1.2)	3 (4.6)	0	3 (6.0)	2 (12.5)				
D/D	1 (0.4)	0	0	0	0				
Sum *O/O*	7 (2.8)	5 (7.4)	0	5 (10.0)	3 (18.8)	3.2 (0.8–12)	0	4.7 (1.1–19)	9.6 (1.3–54)
Total	250 (100)	68 (100)	18 (100)	50 (100)	16 (100)				

A is the designation for wild type. Allele *O* is used as the common designation for the variant alleles *B* (codon 54), *C* (codon 57), and *D* (codon 52). Values are given as absolute numbers and percentages in brackets. OR: odds ratio with 95% confidence interval. *p = 0.09. †p = 0.02. ‡p = 0.004 (chi-squared for trend).

to controls. Of the 35 patients with *A/A*, 18 patients (51%) had *Y/Y*, 16 patients (46%) had *X/Y*, and one patient (2.9%) was homozygous for *X*, compared with 71 (45%), 73 (47%), and 12 (7.6%), respectively, of controls (chi-squared = 1.2, 2 df, p = 0.55). Among patients with *A/O*, 19 patients (68%) did not carry the *X* allele, while 9 patients (32%) did. The corresponding numbers in the controls were 52 (60%) and 34 (40%), respectively (p = 0.64, Fisher's exact test). Figure 1 shows how serum MBL is regulated by the MBL structural alleles and the downregulating *X* promoter allele.

The distribution of HLA-DRB1 genotypes in the patient material was DR4/DR4 (n = 6), DR4/DR1 (n = 2), DR1/DR1 (n = 0), DR4/DRX (n = 12), DR1/DRX (n = 9), and other genotypes (n = 39, data not shown). The corresponding numbers in the 192 controls were 6, 5, 2, 57, 36, and 86. The distributions did not differ with statistical significance (chi-squared = 9.5, 5 df, p = 0.09), nor did they differ when selecting patients who had RA or erosive RA (data not shown). DR1 and/or DR4 were present in 29 of the 68 patients (43%) and in 106 of the 192 controls (55%) (p = 0.10, Fisher's exact test). DR1 and/or DR4 were present in 22 patients with RA and 6 patients with erosive RA, which did not differ significantly from remaining patients and controls (p > 0.20 for all comparisons).

Association of MBL polymorphisms with clinical and laboratory features at inclusion. The duration of symptoms prior to inclusion was significantly longer in the *XA/O + O/O* group (median 9 mo) compared to the rest of the patients (median 4 mo) (p = 0.002). This difference was mainly carried by the *O/O* patients (Table 1). Sex was not associated with MBL polymorphisms. The patients in the *XA/O + O/O* group tended to be younger at the time of inclusion than those in the *A/A + YA/O* group (p = 0.07). Patients belonging to the *XA/O + O/O* group also tended to have fewer swollen joints and lower ratings on physician global assessment compared to the rest of the patients. The number of tender joints, HAQ score, and patient's own assessment of pain disease activity were not significantly related to the MBL genotype.

The prevalence of IgM RF was 13, 12, and 4 in the *A/A* (37%), *A/O* (43%), and *O/O* (80%) groups, respectively. As shown in Table 1, the absolute values of IgM RF were highest in the *O/O* group (p = 0.05) and in the *XA/O + O/O* group (p = 0.02). CRP was significantly higher in the *O/O* group compared to the rest of the patients (p = 0.02).

Patients with RA. Forty-one patients had RA at the time of inclusion and 9 more developed RA during the one year observation period. All 5 patients who were homozygous for *O* alleles (*O/O*) had RA at inclusion. As can be deduced from Table 3, the risk of having RA in this cohort given the presence of *O/O* was increased by a factor of 1.8 at inclusion and 1.4 after one year, but these findings were not statistically significant. Patients belonging to the *XA/O + O/O* group did not have a significantly increased risk of RA. Nor

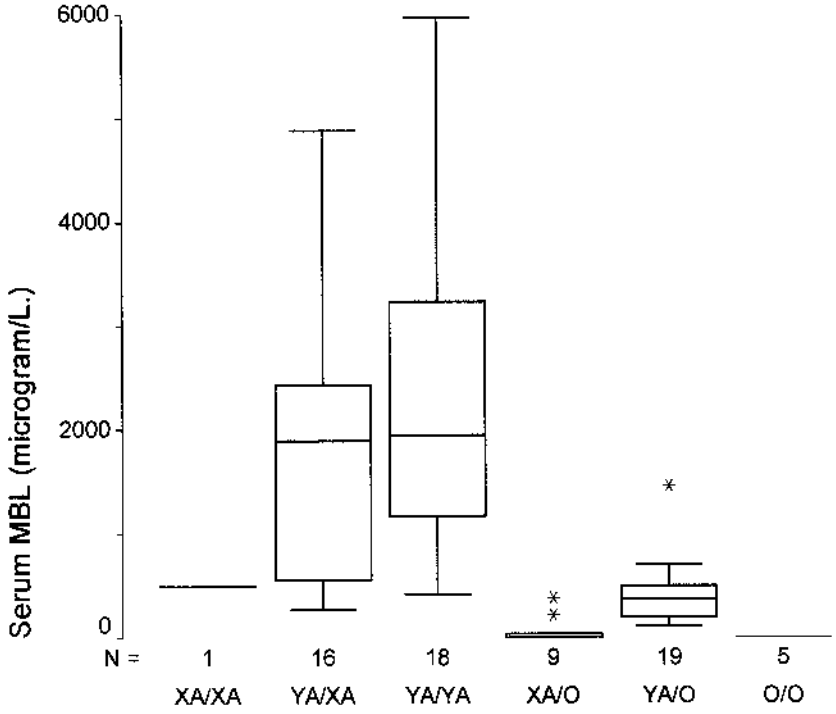

Figure 1. Serum mannose-binding lectin (MBL) concentrations in relation to MBL structural alleles and to the down-regulating MBL promoter allele in position –221 (X versus Y alleles) in patients with early polyarthritis. The detection limit of the assay is 20 μg/l. The box-plot shows median, quartiles, range and extreme values (*: cases with values more than 3 interquartile ranges from the upper or lower edge of the box).

were the HLA-DRB1 alleles significantly related to RA (only DR4 and SE shown in Table 3).

Patients with erosive RA. Thirteen patients had erosive RA at the time of inception and 3 more developed erosive disease after one year. The 16 patients who had erosive RA equal 24% of the whole cohort and 32% of the patients who had RA. Median duration of symptoms prior to inclusion in patients with nonerosive and erosive RA was 3 and 6 months, respectively (p = 0.24). One patient with RA and MBL genotype *O*/*O* was excluded in this analysis as she died prior to radiographic followup. Three of 4 *O*/*O* patients available for analysis in this cohort had erosive RA, which equaled a significant risk increase by a factor of 4.7 (p = 0.02) at inclusion and 3.6 (p = 0.04) after one year of observation of the cohort (Table 4). In patients with the shared epitope these figures were 18.8 (p = 0.004) and 6.3 (p = 0.02), respectively. The distinction between the *YA*/*O* + *A*/*A* and *XA*/*O* + *O*/*O* groups did not reveal an association with erosive RA. No HLA-DRB1 tissue types were individually associated with erosive RA (only DR1 and DR4 shown in Table 4).

DISCUSSION

Our study is the first to report MBL polymorphisms in an inception cohort of patients with early polyarthritis. The cohort represents a fairly small sample size but was intensely examined with regard to the presence and development of RA and erosive outcome. We observed a nonsignificant increase in MBL variant alleles located in the structural part of the gene compared with healthy controls. However, after one year of followup it was revealed that among those who had RA or erosive RA, the frequency of MBL variant alleles was significantly increased compared with healthy controls. This increase in the prevalence of MBL variant alleles was carried by patients with the *O*/*O* genotype. The frequency of *O*/*O* in the controls was 2.8%, but increased to 10.0% and 18.8% in patients with RA and erosive RA, respectively. The distribution of the downregulating MBL promoter allele, *X*, did not differ between patients and controls, as reported[23]. It has been demonstrated that patients with RA have an increased prevalence of undetectable MBL in serum[22], and analysis of MBL polymorphisms has shown a nonsignificant trend towards an increased prevalence of MBL variant alleles in RA patients[24]. These findings are not corroborated by 2 other studies on patients with RA. A British study of serum MBL in sera selected due to the presence of IgG RF[34] is clearly flawed with a selection bias that makes the study difficult to interpret. In another study of MBL variant alleles in RA, only the codon 54 variant (*B* allele) was analyzed[35]. A recent

Table 3. Prevalences and relative risks with 95% confidence intervals (CI) of rheumatoid arthritis during a one-year followup in a cohort of 68 patients with early polyarthritis according to mannose-binding (MBL) and HLA-DRB1 genotypes.

		No. of Patients at Inclusion	Patients with RA at inclusion			Patients with RA at followup		
			No.	(%)	p*	No.	(%)	p*
MBL genotype	*A/A+ A/O*	63	36	(57)		45	(71)	
	O/O	5	5	(100)	= 0.07	5	(100)	= 0.20
Expanded MBL	*YA/O+A/A*	54	31	(57)		39	(72)	
genotype	*XA/O+O?O*	14	10	(71)	= 0.16	11	(79)	= 0.25
DR4	Negative	48	27	(56)		34	(71)	
	Positive	20	14	(70)	= 0.13	16	(80)	= 0.18
DR1 and/or DR4	Negative	39	22	(56)		28	(72)	
	Positive	29	19	(66)	= 0.15	22	(76)	= 0.21

*Two-sided Fisher's exact test.

Table 4. Prevalences and relative risks with 95% confidence intervals (CI) of erosive rheumatoid arthritis (eRA) during a one-year followup in a cohort of 68 patients with early polyarthritis according to mannose-binding lectin (MBL) and HLA-DRB1 genotypes.

		No. of Patients at Inclusion*	Patients with eRA at inclusion			Patients with eRA at followup		
			No.	(%)	p**	No.	(%)	p**
MBL genotype	*A/A+ A/O*	63	10	(16)		13	(21)	
	O/O	4	3	(75)	= 0.02	3	(75)	= 0.04
Expanded MBL	*YA/O+A/A*	54	10	(19)		13	(24)	
genotype	*XA/O+O?O*	13	3	(23)	= 0.27	3	(23)	= 0.28
DR4	Negative	47	10	(21)		12	(26)	
	Positive	20	3	(15)	= 0.23	4	(20)	= 0.23
DR1 and/or DR4	Negative	38	9	(24)		10	(26)	
	Positive	29	4	(14)	= 0.15	6	(23)	= 0.20

*One patient with very active, seropositive RA and genotype *O/O* was excluded in this analysis as she died prior to radiographic followup.
**Two-sided Fisher's exact test.

larger Chinese study did, however, find an increased frequency of codon 54 mutation in patients with RA compared to healthy controls[36]. Tested alone, the frequency of the *B* allele did not differ significantly from the controls in our study, which underlines the necessity of analyzing for all the known MBL variant alleles to obtain a full and more sensitive picture.

In this and other studies[16,20,23] it appears that homozygosity for the structural variant alleles (*O*/*O*) may have a more severe disease outcome than those with the *XA*/*O* genotype, even though both groups lack MBL. However, this does not necessarily mean that individuals carrying these genotypes are devoid of MBL since the detection limit of our assay is 20 μg/l. By molecular characterization using techniques that are more sensitive, we can show that those with the *XA*/*O* genotype indeed may have low concentrations of functional MBL of high molecular weight in the blood. By contrast, those with the *O*/*O* genotype may only have dysfunctional low molecular weight material of MBL, probably explaining this discrepancy (Garred, *et al*, unpublished data).

Several reports on MBL variant alleles in RA are based on patient populations that are either retrospectively identified or consist of current clinic attenders, which may introduce a left censorship bias[37]. We feel that the present study overcomes several of these important caveats and indicates that the MBL gene polymorphisms are susceptibility markers of RA. Also, MBL deficiency is a weak susceptibility marker in systemic lupus erythematosus[21,38,39] and increases the risk of rheumatologic autoimmune disorders in patients with chronic granulomatous disease[40]. The mechanism for this susceptibility effect is unknown. It may be speculated that MBL deficiency leads to a decreased activity of the innate function of the immune apparatus, which might increase presentation of exogenous antigens to the host. Such a lifelong alteration in epitope presentation may alter the immunologic repertoire. Molecular mimicry is one mechanism by which presentation of exogenous antigens immunologically similar to self-antigens may induce an immune response against self-antigens. The loss of self-tolerance may lead to increased susceptibility to autoimmunity and subsequently to manifest autoimmune diseases[41]. In our study, patients in the MBL deficient group *XA*/*O* + *O*/*O* had a median age of 40 years at inclusion in contrast to 54 years in the rest of the patients. This difference in age only reached borderline statistical significance in this study, but similar results have been documented in a larger cohort of patients with RA[24]. In this larger study, a gene dose effect was described as well. These findings indicate a protracting, but not protective, effect of MBL on the development of RA and support the described hypothesis. Early onset of RA in MBL deficient individuals may increase the time in which the disease interacts with the host, leading to increased cumulative damage in the individual patient. In this way, susceptibility characteristics of the MBL gene might modify the disease development.

Recent findings point to more specific disease modifying effects of MBL variant alleles. Graudal, *et al* showed that RA patients with low serum levels of MBL had a high annual increase in radiographic destruction score[22]. Later, in a retrospective study of a cohort of 140 patients with RA, Graudal, *et al* described a highly increased risk of severe erosive outcome in patients with MBL variant alleles compared to the rest of the patients[23]. In that study, patients were identified during the period 1966 to 1978, and more than half the cohort had died when studied in 1997. These circumstances might cause a selection bias in the study, which may explain why only 5 patients (3.6%) carried the MBL variant allele in the homozygous state (*O*/*O*) compared to 10% of patients with RA in our study. However, the studies by Graudal, *et al* and a recent Chinese report by Ip, *et al*[36] corroborate our findings of a highly increased risk of erosive outcome in RA when carrying MBL variant alleles. The risk ratio for MBL homozygous variant alleles and erosive RA presented in this study probably represents a conservative estimate. One patient with RA and the MBL genotype *O*/*O* who died prior to radiologic followup had very high serum levels of CRP and IgM RF and a high swollen joint count, making it highly probable that the patient would have developed erosive disease during the planned followup. Further, our findings suggest that the effect of *O*/*O* on erosive disease is particularly pronounced in patients with the shared epitope. Such a synergistic effect would be in line with the hypotheses. However, due to the small number of patients in this subgroup of interest we do not feel this is a major finding in this study.

The mechanism for this adverse effect of MBL variant alleles is still unknown but may include immunomodulating effects of MBL. The binding of MBL to immune complexes rich in agalactosyl IgG (IgG-G0) known to be present in large quantities in patients with RA may activate the complement system and thus enhance inflammation[42]. In this context, MBL deficiency would be expected to have a reducing effect on inflammatory activity. On the other hand, MBL deficiency may be expected to be associated with defective clearing of apoptotic material and immune complexes as seen in individuals deficient in C1q[43,44], a molecule with characteristics very similar to those of MBL[5]. Our finding of higher serum levels of IgM RF in MBL deficient patients than in MBL competent patients supports this hypothesis along with higher CRP values. The proinflammatory effect of reduced clearing of immune complexes may thus override the possible above-mentioned beneficial effect of MBL deficiency in patients with RA. Further, if maintenance of inflammatory activity in chronic arthritis is partially dependent on an exogenous antigen drive of an

arthropathic cross reaction[45,46], MBL deficiency may facilitate such a mechanism.

Our findings of low physician assessed disease activity in MBL deficient patients and the lack of differences in the remaining ACR activity measures compared with MBL competent patients contrasts with the increased IgM RF and CRP, as well as the poor radiographic outcome in these patients. The MBL deficient patients had the longest duration of symptoms prior to inclusion. To this end, it has been argued that the lag time from onset of symptoms to medical encounter may result in diagnosis of RA, when it may be too late to initiate adequate treatment[47]. However, in the present cohort, most cases of erosive disease had already developed at inception and the duration of symptoms prior to inclusion did not differ significantly between patients with nonerosive and erosive RA. Further, differences in the use of DMARD, which could be prompted by differing clinical pictures at presentation, were not found. The present findings support the above and previously mentioned notion of a 2 edged role of MBL in the pathogenesis of RA[24]. Further, the latest results on anti-tumor necrosis factor-α (TNF-α) blockade therapy reported at the 63rd Annual Scientific Meeting of the ACR in Boston have shown that radiographic progression can be slowed even in the absence of a clinical response[48]. Anti-TNF-α therapy also reduces serum levels of matrix metalloproteinases in patients with RA[49]. These and other findings indicate the presence of chondro- and proteolytic pathways that are regulated by TNF-α[50,51]. In this context, it is of specific interest that MBL has been shown to have modulating effects on monocyte secretion of TNF-α[52,53].

In our study, HLA-DRB1*04 and/or *01 alleles were not associated with RA susceptibility or erosive RA. These findings are in accord with recent large scale epidemiologic cohort studies on early RA that have shown only weak associations between specific HLA-DRB1 alleles and disease susceptibility and no associations to disease severity or progression[3,54,55]. However, such associations are well established findings in several retrospective and hospital based cohorts of patients with RA[56-58], indicating the presence of selection effects that may be conferred by HLA-DRB1*04 and/or *01 alleles.

In spite of a relatively small sample size, we find that this study adds to the body of knowledge pointing at MBL variant alleles to be weak susceptibility markers for RA. Our study also shows that the previously described risk of erosive disease conferred by homozygous MBL variant alleles is not only restricted to RA patients with long disease duration but is also operative in patients with early polyarthritis. These findings, together with the positive association between MBL variant alleles and the increased serum levels of IgM RF and CRP, indicate that MBL is an important factor in the pathophysiology of RA.

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