Mannose-Binding Lectin Gene Polymorphisms Are Associated with Disease Activity and Physical Disability in Untreated, Anti-Cyclic Citrullinated Peptide-Positive Patients with Early Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To study the association between polymorphisms in the mannose-binding lectin gene (*MBL2)* and disease activity, physical disability, and joint erosions in patients with newly diagnosed rheumatoid arthritis (RA).

Methods. Patients with early RA (n = 158) not previously treated with disease modifying antirheumatic drugs, participating in a treatment trial (CIMESTRA study) were examined at inclusion for *MBL2* pooled structural genotypes (O/O, A/O, A/A), regulatory *MBL2* promoter polymorphism in position –221 (XX, XY, YY), anti-cyclic citrullinated peptide 2 antibodies (anti-CCP2), disease activity by Disease Activity Score-28 (DAS28 score), physical disability by Health Assessment Questionnaire (HAQ) score, and erosive changes in hands and feet (Sharp-van der Heijde score).

Results. Eight patients were homozygous *MBL2* defective (O/O), 101 belonged to an intermediate group, and 49 were *MBL2* high producers (YA/YA). Anti-CCP was present in 93 patients (59%). High scores of disease activity, C-reactive protein-based DAS28 (p = 0.02), and physical disability by HAQ (p = 0.01) were associated with high *MBL2* expression genotypes in a gene-dose dependent way, but only in anti-CCP-positive patients. At this early stage of the disease there was no association with erosion score from radiographs.

Conclusion. The results point to a synovitis-enhancing effect of MBL in anti-CCP-positive RA, whereas such an effect was not demonstrated for joint erosions. (First Release March 1 2009; J Rheumatol 2009;36:731–5; doi:10.3899/jrheum.080846)

Key Indexing Terms:
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Rheumatoid arthritis (RA) is a systemic disease with autoimmune features characterized mainly by chronic synovitis, physical disability, and particularly in patients with anti-cyclic citrullinated peptide (anti-CCP) antibodies by progressive joint destruction unless appropriate therapy is instituted[1,2]. Development of anti-CCP-positive RA has been shown to be associated with specific genetic and environmental risk factors such as shared epitope and smoking, among others, indicating a pathogenesis that differs from anti-CCP-negative RA[3,4].

Mannose-binding lectin (MBL) is a liver-derived component of the innate immune system that may bind to various sugar motifs and thereby activate complement through MBL-associated serine proteases. Serum concentrations of MBL mainly reflect the expression of the *MBL2* gene in the liver[5,6]. Subjects homozygous or compound-heterozygous for defective structural *MBL2* alleles produce dysfunctional low molecular weight MBL with little or no apparent biological function[7], whereas subjects with the extended YA/YA haplotype have the highest MBL-producing potential. Studies have investigated the role of MBL in RA; some found that low levels of MBL are associated with erosive disease in RA[8,9], whereas others have not[10]. On the other hand, recent studies have found that increased serum levels of MBL are associated with increased risk of developing RA[11] and of developing ischemic heart disease when having RA[12]. If an interaction between MBL and anti-CCP antibodies to some extent is associated with a particular presentation of RA, then the divergent MBL association results noted above could partly be attributable to varying frequencies of anti-CCP positivity in the RA populations studied.

The objective of our study was to establish whether polymorphisms of *MBL2* and promoter genes are associated with anti-CCP autoantibody status, disease activity, physical disability, and erosive disease at presentation in early untreated RA.

MATERIALS AND METHODS

Patients. Patients in this study derived from an investigator-initiated, multicenter, randomized, double-blind controlled treatment trial of 160 consecutive patients with early active RA (the CIMESTRA study)[13]. At inclusion, all participants fulfilled the American College of Rheumatology 1987 revised criteria for RA[14], were disease-modifying antirheumatic drug (DMARD)-naive, and had active known disease of < 6 months' duration with at least 2 swollen joints. In 2 patients, *MBL2* genotyping or determination of anti-CCP was not available. Baseline characteristics of the 158 patients studied are presented in Table 1.

All patients gave written informed consent to participate, and the protocol was approved by the national health authorities and ethics committees in all 5 participating counties. The trial was performed in accord with the Declaration of Helsinki and the International Conference on Harmonisation 1996 revised Guidelines for Good Clinical Practice in the European Community.

Clinical, radiographic, and laboratory assessments at inclusion. Disease activity was assessed by the Disease Activity Score, using C-reactive protein (CRP) and a 28-joint score (DAS28)[15]. Physical disability was assessed by means of the Danish version of the Stanford Health Assessment Questionnaire (HAQ)[16]. Radiographic status was determined by conventional radiographs of the hands, wrists, and forefeet scored by a senior musculoskeletal radiologist using the Sharp-van der Heijde method[17]. Anti-CCP IgG antibodies were determined by a second-generation ELISA (Immunoscan RA kit; Euro-Diagnostica AB, Malmö, Sweden) in accord with the manufacturer's instructions using a cutoff at 25 U/ml. Serum CRP (mg/l) was measured using standard laboratory methods.

MBL2 genotyping. Genomic DNA was isolated from EDTA-preserved blood cells, using a QIAamp Maxi Kit (Qiagen, Chatsworth, CA, USA) in accord with the manufacturer's instructions, and stored at –20°C before genotyping. *MBL2* alleles (O/O, O/A, A/A) and promoter polymorphisms at position –221 (X/X, X/Y, Y/Y) were detected as described[5,6]. Patients were stratified into those with O/O genotype (dysfunctional) and YA/YA extended haplotype (high-producing), and a third group consisting of the remaining haplotypes (XA/O, YA/O, XA/XA, and YA/XA) with an intermediate MBL-producing potential.

Statistical analysis. Nonparametric analyses included the Mann-Whitney test, Jonckheere-Terpstra test for trend, and the Spearman test for correlation analysis. Statistical significance was defined as $p < 0.05$.

RESULTS

The demographic and basic features of the patients, including disease activity (DAS28), physical disability (HAQ score), and degree of erosive changes (Sharp-van der Heijde score), are described in Table 1. Ninety-six patients (60.8%) had *MBL2* genotype A/A, 54 (34.2%) had genotype A/O, and 8 (5.1%) had genotype O/O. With regard to the *MBL2* promoter allele X/Y, 95 (60.1%) were homozygous for the high expression allele Y, and 49 (31.0%) had the extended genotype YA/YA. Positive anti-CCP status was observed in 93 (58.8%) RA patients and was not statistically significantly related to demographic or basic features or *MBL2* genotype distribution. However, the proportion of patients with the extended YA/YA genotype was relatively the highest in anti-CCP-positive patients (Table 1).

Associations between the various outcome measures were tested, and correlation analyses showed a high correlation between scores for disease activity and physical disability (rho = 0.61, $p < 0.0001$), a low to moderate correlation between scores for disease activity and erosive status (rho = 0.18, $p = 0.03$), and no correlation between scores for physical disability and erosive status (rho = 0.01, $p = 0.87$).

Scores for disease activity and physical disability increased with increasing MBL-producing potential of the 3 groups of anti-CCP-positive patients that were stratified according to *MBL2* genotype O/O, the intermediate group, and YA/YA, respectively. The largest group difference for both disease activity and physical disability was seen between the homozygous-defective patients and the remaining patients. These effects were not observed in anti-CCP-negative patients, and there was no association between *MBL2* genotype and scores for erosive status irrespective of anti-CCP status (Table 2).

DISCUSSION

A main finding of this study was the dose-dependent association between *MBL2* expression potential (indicated by

Table 1. Demographic, clinical, and paraclinical characteristics including disease activity (DAS28), erosive status (Sharp-van der Heijde score), and physical disability (HAQ score) of 158 patients with early untreated RA by anti-CCP status. Data are median (range).

	All Patients, n = 158	Anti-CCP-positive, n = 93	Anti-CCP-negative, n = 65	p†
Age, yrs	53 (20–75)	53 (22–75)	53 (20–73)	0.92*
Women (% of patients)	105 (66.4)	59 (63.4)	46 (70.8)	0.34**
Duration since first symptom, wks	14 (6.3–27)	15 (6.3–26)	14 (6.3–27)	0.84*
Disease activity	5.5 (2.2–8.4)	5.6 (2.2–8.4)	5.5 (2.4–7.9)	0.86*
Erosive status	3 (0–37)	4 (0–37)	2 (0–26)	0.11*
Physical disability	1.0 (0–2.9)	1.0 (0–2.9)	0.75 (0–2.4)	0.10*
MBL2 genotypes				0.17***
Homozygous-defective, 0/0 (%)	8 (5.1)	5 (5.4)	3 (4.6)	
Intermediate group (%)	101 (63.9)	54 (58.0)	47 (72.3)	
High producers, YA/YA (%)	49 (31.0)	34 (36.6)	15 (23.1)	

† Anti-CCP-positive vs anti-CCP-negative patients. * Mann-Whitney test, ** Fisher exact test, *** 2 × 3 chi-square test.

Table 2. Disease activity (DAS28), erosive status (Sharp-van der Heijde score), and physical disability (HAQ score) by MBL2 genotype and anti-CCP status in patients with early untreated RA stratified by anti-CCP status (positive, n = 93; negative, n = 65). Data are median (range).

	Homozygous-defective 0/0, n = 8	Intermediate Group, n = 101	High Producers YA/YA, n = 49	p*
Disease activity				
Anti-CCP-positive	4.6 (3.2–5.7)	5.5 (2.2–7.9)	5.7 (2.5–8.4)	0.02
Anti-CCP-negative	5.8 (5.5–6.4)	5.5 (2.4–7.8)	5.6 (3.8–7.9)	0.89
Erosive status				
Anti-CCP-positive	6 (0–24)	3 (0–24)	4 (0–37)	0.86
Anti-CCP-negative	3 (0–6)	1 (0–26)	2 (0–24)	0.98
Physical disability				
Anti-CCP-positive	0.25 (0.13–0.63)	1.0 (0–2.9)	1.1 (0–2.6)	0.01
Anti-CCP-negative	1.1 (0.4–1.1)	0.88 (0–2.4)	0.50 (0–2.0)	0.19

* Jonckheere-Terpstra test for trend.

genotypes) and disease activity and physical disability in anti-CCP-positive patients with RA before initiation of DMARD treatment. This finding is new, but is in accord with previous findings of increased serum concentrations of MBL in patients with RA[11].

The high correlation between scores of disease activity and physical disability in our study may explain why our observation of an association between high-producing *MBL2* genotypes and disease activity was paralleled by a similar association with HAQ score. This information may seem redundant; however, physical disability represents another dimension of the disease burden in RA in addition to disease activity and chronic irreversible joint changes. These 3 indicators may be interrelated to some extent[18], but in this study of patients with early RA, joint changes were not prominent and were not associated with physical disability.

Due to the possibility that increased serum levels of MBL may reflect an acute-phase reaction, we focused on *MBL2* genotypes. The association between *MBL2* genotypes and disease activity in our study is particularly driven by the relatively small group of patients that were homozygous-defective and were characterized by the lowest median values of disease activity and HAQ scores. As the number of patients with homozygous defect in this study was small, rigid conclusions may be difficult to obtain. However, statistical significance was obtained in the tests used, and the findings may thus indicate that clinically detectable inflammation in RA is at least partly dependent on activation of the MBL-dependent lectin complement pathway.

Glycosylation changes of the IgG Fc region in patients with RA result in a reduced content of oligosaccharide chains terminating in galactose, leading to exposure of *N*-acetylglucosamine. This glycosylation variant, agalactosyl IgG (IgG-G0), is prevalent in RA and is associated with poor prognosis[19]. *In vitro* studies have shown that MBL may bind to the exposed *N*-acetylglucosamine of agalactosyl IgG and thus activate complement[20] through the lectin pathway. Further, a recent study has demonstrated how the combination of high serum levels of both MBL and IgG-G0 confer

an increased risk of ischemic heart disease in patients with RA[12]. Conversely, our data may also suggest that low *MBL2* expression may have protective effects in disease states. This notion is supported by reports from studies of arteriosclerosis[21,22] and diabetes mellitus[23].

However, the associations noted here were only recorded among anti-CCP-positive patients. This may reflect significant differences in the pathogenesis of anti-CCP-positive and anti-CCP-negative RA. This view is supported by completely different genetic and environmental factors being associated with risk of developing anti-CCP-positive and anti-CCP-negative RA[3]. Further, antibodies against citrullinated proteins (ACPA) have been shown to enhance signs of inflammation and tissue injury in collagen-induced arthritis[24]. However, this mechanism seems to depend on factors absent in mice deficient for complement receptor Cr2[25], which plays a key role in the activation of the classical complement pathway but also the lectin pathway of complement activation. The significance of complement activation in the pathogenesis of RA, irrespective of pathway, has been recognized for more than 30 years[26], and has recently been substantiated on the gene level by the identification of *TRAF1*-C5 as a risk locus for anti-CCP-positive RA[27]. However, the mechanism by which anti-CCP and MBL may interact in a proinflammatory way is not known. Galactosylation of IgG is reduced in RA[20] and is inversely correlated with disease activity[28]. This could theoretically also apply for IgG ACPA, which in this cohort of patients with active untreated RA would implicate a relatively large proportion of IgG-G0 anti-CCP. Complexes of IgG-G0 ACPA and citrullinated proteins in the synovial tissue of RA patients may constitute a source of MBL ligands, resulting in complement activation through the lectin pathway. However, this remains to be shown and is contrasted by recent findings, although in mice, of a relatively limited C3 activation by MBL binding to IgG-G0[29] and by unimpaired activity of IgG-G0 antibodies in MBL-null mice[30].

Although our study comprised only a relatively small number of homozygous-defective RA patients, the results support the notion that MBL-dependent lectin complement activation was involved in the pathogenesis of joint inflammation in the anti-CCP-positive subset of patients. Such an association was not observed for scores of radiographically confirmed erosive changes in our patients who had newly diagnosed RA. In contrast, previously reported associations between MBL status and erosive disease in both early and established RA have tended to be with states of MBL deficiency[8,9]. To this end, the subgroup of patients in our study with the highest median erosion score were those who were anti-CCP-positive and MBL homozygous-defective. The low number of patients in this group and the limited erosion involvement in our population introduces a large type 2 error. These and previous findings do not support a role of activation of the lectin complement pathway in the pathogenesis of the erosive component of early RA, but may suggest a more complex role where the lectin pathway may be partly involved in the generation of the general systemic inflammatory response in these patients.

Together, these findings do not support a role of activation of the lectin complement pathway for the development of joint erosions in early RA. By contrast, high *MBL2* expression capacity, with the potential to activate complement via the lectin pathway, may be one component of a large mosaic of mechanisms involved in local and systemic rheumatoid inflammation.

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