Patients with Rheumatoid Arthritis Have Higher Levels of Mannan-Binding Lectin Than Their First-degree Relatives and Unrelated Controls

SAEDIS SAEVARSDOTTIR, KRISTJAN STEINSSON, GERDUR GRONDAL, and HELGI VALDIMARSSON

ABSTRACT. ***Objective.*** Mannan-binding lectin (MBL) is present in serum and synovial fluid; its levels vary widely, and the variations are strongly associated with polymorphisms in the *MBL2* gene. Studies have compared MBL in patients with rheumatoid arthritis (RA) and in unrelated controls, but the findings have been contradictory. In the first family-based study, we compared MBL levels in patients with RA to population controls and also to their nonaffected first-degree relatives, who may be regarded as optimal controls because of less genetic variation.

Methods. Serum levels of MBL and rheumatoid factor were analyzed in 210 patients with RA and 406 of their first-degree relatives from 74 extended families. Population controls for MBL levels were 330 randomly selected adult Icelanders.

Results. Patients with RA had higher MBL levels in serum (median 1553 μg/l) than their first-degree relatives (1073 μg/l; $p = 0.003$) and the unrelated controls (938 μg/l; $p < 0.0001$). No association was found between MBL and rheumatoid factor.

Conclusion. Patients with RA had markedly higher MBL levels than their close relatives and controls, indicating that high MBL may predispose to RA. As MBL has been shown to bind potential arthritogenic agents including modified immunoglobulins, cellular debris, and microorganisms, our findings suggest that high MBL could trigger complement mediated inflammation within joints. (First Release June 15 2007; J Rheumatol 2007;34:1692–4)

Key Indexing Terms:
MANNAN-BINDING LECTIN | RHEUMATOID ARTHRITIS
RHEUMATOID FACTOR | EXTENDED FAMILY | COMPLEMENT

Rheumatoid arthritis (RA) is believed to result from a complex interplay between genetic and environmental factors, and each predisposing factor may only be associated with moderately increased risk. Large study groups with carefully selected controls are therefore needed to identify individual susceptibility factors, and first-degree relatives may, due to less genetic variation, be regarded as optimal controls in this respect[1].

Mannan-binding lectin (MBL) is a sugar-binding complement-activating protein that is present in both serum and synovial fluid. MBL can bind to various potential pathogenic agents in RA, including microorganisms, immunoglobulins, and cell debris[2]. MBL levels are stable for each individual over time but vary widely (0–10,000 μg/l) between individuals[3]. The MBL protein is coded by the *MBL2* gene on chromosome 10q11.2-21. A few polymorphisms have been identified in the gene, which minor alleles are strongly associated with MBL levels below 1000 μg/l[4].

Several studies have analyzed MBL in RA. Some reports have suggested an increased frequency of low MBL levels or associated polymorphisms in patients with RA compared to unrelated controls[5-7], while others have not revealed any such association[8-12], and a recent study even showed opposite findings[13]. To our knowledge, this is the first family-based study on MBL in RA.

MATERIALS AND METHODS

Our study was carried out in accord with the Helsinki Declaration, and was approved by the Ethics Committee of the University Hospital in Iceland and the Icelandic Computer Database Committee. All participants provided informed consent.

A total of 210 patients with RA (169 women, 41 men) derived from 74 families were analyzed (194 patients from 58 multicase families and 16 sporadic cases). All patients fulfilled ≥ 4 American College of Rheumatology classification criteria for RA[14]. Available family members were evaluated by rheumatologists through a questionnaire, clinical examination, and structured review of medical records. This included 406 non-RA first-degree relatives, of whom 41 were relatives of sporadic cases. Ninety-eight patients were

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rheumatoid factor (RF) positive and 83 had erosive disease. Population controls for MBL levels were 330 adult Icelanders[12]. As information about age and sex was not available for all the population controls, the cases and controls were not matched, but these variables have not been shown to influence MBL levels after early childhood.

MBL was measured by a sandwich-ELISA system[12]. No evidence was found for interference by RF in this assay[12]. RF was measured both by agglutination using the RAPA kit (Fujirebio Inc., Tokyo, Japan) and by an isotype (IgM, IgA, IgG RF)-specific ELISA.

Statistical analysis. The Mann-Whitney rank sum test was used to compare MBL serum levels between 2 groups and Fisher's exact test for frequency distribution between groups. The Spearman correlation was used for correlation between 2 variables. Statistical analysis was performed using the SigmaStat and GraphPad (Prism4) software. All tests were 2-sided and the level of significance was set at $p < 0.05$.

RESULTS

As shown in Figure 1, the RA patients had markedly higher MBL levels (median 1553 µg/l) than their first-degree relatives (1073 µg/l; $p = 0.003$), and the patients with RA also had higher MBL levels than the population-based control group (938 µg/l; $p < 0.0001$). This applied to patients from both the multicase and single-case families and these 2 groups were therefore pooled. Those patients with RA (n = 21) who did not have available first-degree relatives were excluded from our study, but it should be noted that they had a distribution of MBL levels similar to the remaining patients (1699 µg/l). A cutoff level of 1000 µg/l distinguishes well between individuals with and those without polymorphisms in the *MBL2* gene[4]. Of the patients with RA, 61% had MBL levels above 1000 µg/l compared to 52% of first-degree relatives and 49% of the controls [patients vs relatives: odds ratio (OR) 1.46, 95% confidence interval (CI) 1.04–2.04; patients vs controls: OR 1.60, 95% CI 1.13–2.27). The findings were similar in men and women.

No association was observed between MBL levels and elevated RF in the patients with RA [RAPA agglutination and isotype-specific ELISA (IgM, IgA, and IgG RF), data not shown].

DISCUSSION

This is to our knowledge the first family-based and to date also the largest study analyzing whether MBL may influence RA susceptibility. Contrary to most previous case-control studies[5-13], the patients with RA had markedly higher MBL levels than their first-degree relatives and population-based controls. As RA is believed to result from complex gene-environmental interactions, comparison with first-degree relatives may help to reduce the confounding effect of heterogeneous genetic and environmental background.

Although MBL levels of individuals have been shown to be very stable over decades[3], we cannot exclude that higher MBL levels in the patients with RA might partly be due to acute-phase reaction, as MBL may transiently increase slightly during inflammatory responses. However, only a weak correlation has been observed between MBL and inflammation as measured by erythrocyte sedimentation rate[5]. Moreover, studies of patients with RA have not indicated significant fluctuation in MBL levels over time[5,6,12], and patients with RA[6] or systemic lupus erythematosus[4] have, further, had similar MBL levels compared to healthy controls with corresponding MBL genotypes. Our findings also agree with a recent case-control study, in which patients with RA had decreased frequency of the genetic polymorphisms associated with low MBL levels[13].

Conflicting reports on MBL and the risk of RA may, at least partly, be due to differences in patient cohorts. There has been more consensus about association between low MBL and disease severity[5-8,11,12,15], which our study was not designed to evaluate. We did not observe an association between low MBL and positive IgA and/or IgM RF. This is in contrast to previous studies on early RA by our group and oth-

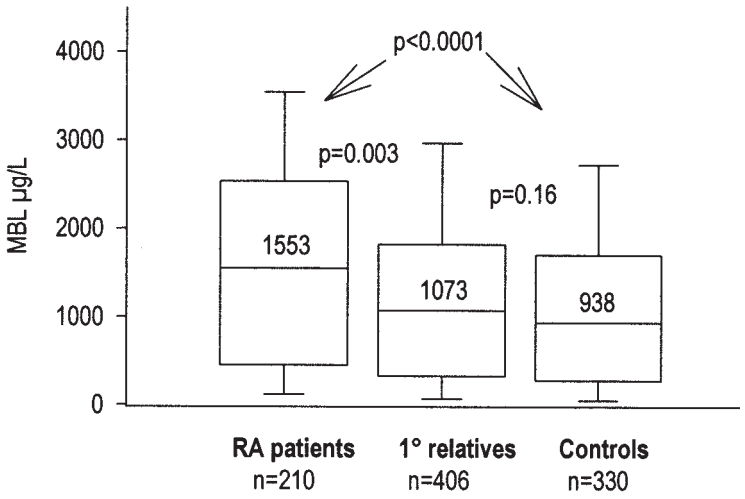

Figure 1. MBL serum levels in patients with RA (n = 210), their first-degree relatives (n = 406), and an Icelandic population-based control group (n = 330). Box plots show median values and interquartiles, and the highest and lowest levels of MBL concentrations are shown by the bars.

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ers[7,12] and such an association may only be detected in patients with early untreated RA.

Our findings indicate that high MBL may trigger RA in predisposed individuals. MBL is present in synovial fluid and it has been shown to bind potential causative agents in RA including microorganisms, cellular debris, and modified immunoglobulins like agalactosylated IgG (IgG-G0), IgA, and glycoforms of IgM[2,16]. In a recent report, high serum levels of both MBL and IgG-G0 were associated with increased risk of ischemic heart disease in patients with RA[17]. It is likely that MBL may bind to ligands within the joint and thereby trigger local complement-mediated inflammation, as has been reported for ischemic tissues[2]. This remains to be studied further.

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