MANNOSE BINDING LECTIN IN SERA AND SYNOVIAL FLUIDS OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Summary: It has been supposed that low levels of mannose binding lectin (MBL) may be involved in protection of rheumatoid arthritis associated with agalactosil forms of GOIgG immunoglobulins found in rheumatoid synovial fluid and lead to enhanced complement activation and joint cartilage damages. In order to examine the possible link between MBL and mediated complement activation, its concentration was measured in sera and synovial fluids of 59 patients classified in moderate and severe activity RA groups and compared with the levels in the same biological fluids of the control group with meniscus lesions. No patients had undetectable or lowered MBL in both RA groups. Mean values were either normal or elevated depending on the severity of the disease as a response to inflammation. This finding does not exclude a possible role of MBL-GOIgG mediated complement activation, but provides no support to mitigating RA severity.

Key words: rheumatoid arthritis, mannose binding lectin, complement, agalactosil IgG

Introduction

Human mannose-binding lectin (MBL) is a macromolecule protein with an apparent Mm from 270 to 650 KD and consists of about 9 to 18 identical subunits-trimers of 32 KD. Each of them has carbohydrate-recognition domain (CRD) at the COOH-terminal, a collagen like domain (CLD) and cystein rich domain at the NH2-terminal portion. CLD associated with 32 KD trimers is a structure unit which can further assemble into higher multimers forming dimeric to hexameric units. So, the gross structure of MBL is remarkably homologous with C1q complement component. MBL is Ca2+ dependent, but unlikely to C1q, functions in antibody as independent host defence against pathogens. Its ability to bind carbohydrate structures (mannose or N-acetylglucosamine) is widely present on yeasts, bacteria and viruses can thereby activate the complement system (1). Deficiency of MBL has been linked to recurrent infections in infants, older children and adults (2–4). The concentration of MBL in sera is largely under genetic control and depends primarily on genetic polymorphism within the first exon of the structural gene (5). A point of mutation at codon 54 gives rise to a mutant allele which has a gene frequency (GF) of 0.11–0.19 in European populations (1, 6). Two other mutant alleles at codon 52 (GF: 0.02–0.05) and 57 (GF: 0.07–0.29) are known and are relatively common in African and Eskimo populations. It has been shown that local activation of complement may play a role in mediating synovial inflammation (7) in rheumatoid arthritis (RA). Synovial fluid (SF) in RA contains high concentrations of immunoglobulin and rheumatoid factors (RF) which, through their ability to form immune complexes, may promote complement activation. In addition, distinctive glycosylation pattern of these synovial immuno-
globulins have been reported, including a raised proportion of IgGs which contain galactose deficient oligosaccharides on side chains in hinge region of CH2 domain (8, 9). Thus, such IgG is called agalactosyl IgG (G0IgG), which terminates with mannose or N-acetylglucosamine becomes accessible for binding to MBL (10) and can mediate opsonization and lectin glucosamine becomes accessible for binding to MBL (10) and can mediate opsonization and lectin glucosamine becomes accessible for binding to MBL (10) and can mediate opsonization and lectin glucosamine becomes accessible for binding to MBL (10) and can mediate opsonization and lectin

**Patients and Methods**

In this multicentric study 59 RF positive sera and SFs were examined in RA patients for the level of MBL and compared with the same biological fluids of a control group of 15 patients with lesion menisci. All RA patients satisfied American Rheumatism Association criteria (16) and were classified in two groups: One with moderate (n=24) disease activity and the other with severe (n=35) disease activity. Activity of RA was determined according to adequate clinical (number of painful and swollen joints, Steinbrocker’s radiographic changes, Ritchie’s articular index, Disease Activity Score) and biochemical (erythrocyte sedimentation rate, fibrinogen, C-reactive protein and serum amyloid A concentration) parameters.

Concentrations of MBL, CRP, SAA, CIC and IgMRF in both fluids were examined by latex immunonephelometry («DADE Behring») method, while IgGRF and IgARF were measured by enzyme immuno («CogentDiagnostics») assays. Before testing all SF samples were pretreated with hyaluronidase (Streptomyces hyalurolyticus).

Statistical analyses were performed using Basic Statistic software for comparison of means (Mann-Whitney U test), standard deviation and data distribution.

**Results**

All patients were IgMRF, IgGRF or IgARF positive, alone or in RF isotype combinations, but there

<table>
<thead>
<tr>
<th>Table I</th>
<th>Comparison of C3c, C4 complement components, MBL and CIC in sera and SFs in three tested groups</th>
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<tbody>
<tr>
<td>CONTROL GROUP N=15</td>
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<tr>
<td>SERUM vs. SF</td>
<td>C3c vs. C3c SF</td>
</tr>
<tr>
<td>C4 vs. C4 SF</td>
<td>0.25 ± 0.09</td>
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<tr>
<td>CIC vs. CIC SF (IU/mL)</td>
<td>1.81 ± 0.76</td>
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<tr>
<td>MBL vs. MBL SF (mg/L)</td>
<td>2.05 ± 1.03</td>
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<tr>
<td>MODERATE ACTIVITY of RA N=24</td>
<td>serum</td>
</tr>
<tr>
<td>C3c vs. C3c SF</td>
<td>1.22 ± 0.37</td>
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<tr>
<td>C4 vs. C4 SF</td>
<td>0.25 ± 0.08</td>
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<tr>
<td>CIC vs. CIC SF (IU/mL)</td>
<td>3.01 ± 2.02</td>
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<tr>
<td>MBL vs. MBL SF (mg/L)</td>
<td>2.56 ± 1.51</td>
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<tr>
<td>SEVERE ACTIVITY of RA N=35</td>
<td>serum</td>
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<tr>
<td>C3c vs. C3c SF</td>
<td>1.33 ± 0.36</td>
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<tr>
<td>C4 vs. C4 SF</td>
<td>0.29 ± 0.13</td>
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<tr>
<td>CIC vs. CIC SF (IU/mL)</td>
<td>7.43 ± 13.4</td>
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<tr>
<td>MBL vs. MBL SF (mg/L)</td>
<td>3.34 ± 1.9</td>
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Abbreviations: CIC – circulated immune complexes; SF – synovial fluid; vs. – versus; N = number of patients; x – arithmetic mean; SD – standard deviation; p – statistical significanucy; r.i. – reference interval; * p < 0.05 vs. adequate value in control group (t – test proportions).
was no correlation ($r < 0.03; p > 0.05$) between RF and CIC levels, with MBL concentration neither in sera nor in SF. MBL sera concentrations were either normal or elevated in about 11% of patients in both MA and SA RA groups. In SF MBL group they were 39%, 44% and 54% lower than sera levels in MA, SA and CG respectively, but these differences were not statistically significant (Table I). Similar results were obtained for C3 and C4 components of complement which were lower in SF (between 55% and 75%). Marked elevation was only found in CIC levels in SF in both RA activity groups in spite to CG; this finding was statistically significant regarding the t-test proportions.

An important result was that no sample of sera of both RA groups had undetectable MBL concentrations. Mean average MBL values in sera, but not in SF, were significantly higher ($p< 0.05$) in SA group compared with MA group, but such differences were not detected in groups MA and CG (Figure 1).

In both cases of the disease activity the SM filtration rate was the same, while CIC concentration in SF was significantly higher in contrast to sera levels. CIC levels in sera and SF compared among SA vs. MA were higher but less than in SA group vs. CG (Table II). No similar differences were found for the level of C3 and C4.

### Discussion

It was expected that patients with RA would have an increased average of MBL concentrations, and conversely, that low MBL concentration would be absent or under-represented. In fact, the obtained results showed statistically higher mean values in sera of SA vs. MA RA groups, but not regarding concentrations in SF. A higher MBL concentration in SF of CG than in sera in RA groups could be explained by a possible more severe SM destruction and, consequently, worse filtration rate in patients with lesion menisci in comparison to SM destruction provoked by inflammation in RA groups.

Results regarding MBL level appeared as a consequence of severe inflammation among the tested groups in relation to MBL as an acute phase reactant.
(APR) production. Moreover, data that none of 59 RA patients had undetectable or significantly lower MBL levels even in MA RA group indicated that the frequency of MBL codon 54 mutation was rather low in RA patients. Result from this study confirmed no hypothesis that in RA, MBL deficiency might mitigate the effects of complement damage mediated by a pathway involving GOIgG, MBL binding and complement activation (10), and so be associated with less severe disease. The others showed no relationship between the presence of codon 54 mutation allele and other indicators of severe RA, such as RF (14). Our previous results revealed a connection between RA severity and high level of different RF isotype combinations (17, 18). In all, this study also suggests that MBL deficiency does not protect against severe RA. Similar conclusions have been reported in a study of MBL protein levels only in sera of RA patients, but were not classified according to the severity of the disease (15).

Unclear aetiopathology of RA up to date, involves a large spectrum of biological molecules with several antigen or autoantigen and adequate antibodies, producing high levels of CIC, which are also important for the severity of RA. The obtained higher CIC level in SF than in serum implies its local production, which could be seen from the result obtained in this study. However, which part of CIC levels belongs to GOIgG complexes and complement activation via MBL is an open question. Complement activation or consumption of C3 and C4 in sera is hardly to detect for the same reason as MBL, because all mentioned molecules belong to APR family. Their production in response to the inflammation possibly masks the known consumption. Some authors, therefore suggest other complement degradation of split products as more relevant for complement activation in RA (19).

In conclusion, the findings presented here do not exclude the putative role of MBL-GOIgG interaction in complement activation in RA, but there is also no evidence of supporting the opinion that such complexes play a major role in the pathogenesis, possible prevention or perpetuation of RA. Measuring MBL as well C3 and C4 in a laboratory routine practice is therefore, time consuming without marked relevant data.

MANOZA VEZUJUĆI LEKTIN U SERUMU I SINOVIALNOJ TEČNOSTI BOLESNIKA SA REUMATOIDNIM ARTRITISOM

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Kratak sadržaj: Pretpostavljalo se da niska koncentracija manoza-vezujućeg lektina (MBL) može biti povezana sa zaštitom prema ispoljavanju reumatoidnog artritisa (RA), zajedno sa gliko formom agalaktozil GOIgG koji je ustanovljen u sinovijalnoj tečnosti. U cilju iznalaženja povezanosti aktivacije komplekta sa RA, određena je koncentracija ovog lektina u serumu i sinovijalnoj tečnosti kod 59 bolesnika koji su razvrstani u grupe sa umerenom i visokom aktivnošću RA. Rezultati su upoređeni sa koncentracijom MBL u oba biološka uzorka iz kontrolne grupe pacijenata sa povredama meniskusa. Nijedan ispitanik nije imao nemerljivu koncentraciju MBL. Srednje vrednosti su bile u okviru fizioloških granica ili pak povišene zavisno od stepena aktivnosti RA, kao proteinski odgovor na proces inflamacije. Ovi rezultati ne isključuju moguću ulogu kompleksa MBL-GOIgG u aktivaciji komplementa, ali ne idu u prilog podrške mišljenju da MBL sudjeluje u ublažavanju jačine ispoljavanja simptoma bolesti.

Ključne reči: manoza vezujući lektin, reumatoidni artritis, complement, agalaktozil IgG
References


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