ELSEVIER

Journal of Autoimmunity 27 (2006) 125–133

journal of autoimmunity

www.elsevier.com/locate/issn/08968411

# Anti-MBL autoantibodies in patients with rheumatoid arthritis: prevalence and clinical significance

Bhawna Gupta [a], Sunil Kumar Raghav [a], Charu Agrawal [a], Ved Prakash Chaturvedi [b], Rakha Hari Das [a], Hasi Rani Das [a,*]

[a] *Institute of Genomics and Integrative Biology, Delhi University Campus, Mall Road, Delhi 110 007, India*
[b] *Department of Rheumatology, Army Hospital, N. Delhi 110 010, India*

Received 23 March 2006; revised 30 June 2006; accepted 1 July 2006

**Abstract**

Occurrence of autoantibodies in patients' sera is the characteristic feature of autoimmune disorders. We assessed the presence of anti-mannose binding lectin (MBL) autoantibodies in the sera of 107 rheumatoid arthritis (RA) patients and 121 control subjects by enzyme immunoassay. Elevated levels of anti-MBL autoantibodies in the sera of RA patients ($P < 0.0001$) was detected for the first time. The ratios of anti-MBL positive in RA patients and controls were respectively 60.7% and 1.65%. Experiments were then designed to understand the functional relevance of these autoantibodies. An inverse correlation of anti-MBL autoantibodies with serum MBL levels ($P = 0.001$) and MBL complex activity ($P = 0.02$) was observed without genetic association between MBL polymorphisms and anti-MBL autoantibody secretion. A significant increase ($P = 0.038$) in the level of anti-MBL autoantibodies was observed in 23 synovial fluid samples in comparison to the serum samples. Moreover, the anti-MBL autoantibodies were found to be more often present in the sera of RA patients (60.75% sensitivity, 98.35% specificity and 0.913 area under the ROC curve) in comparison to the IgM and IgG isotypes of rheumatoid factors (RF). Anti-MBL autoantibodies were still positive in 25.23% RA patients when both the RF isotypes were negative. Also, in RA patients, at all stages of disease activity and joint deformity, anti-MBL autoantibodies were more often present than both the RF isotypes. Therefore, the significant presence of anti-MBL autoantibodies enunciates that anti-MBL autoantibodies might have a diagnostic value; however, more studies are needed to confirm the role of anti-MBL autoantibodies in the diagnosis of rheumatoid arthritis.


*Keywords:* Autoantibodies; Mannose binding lectin; Rheumatoid arthritis; Rheumatoid factor

## 1. Introduction

Rheumatoid arthritis (RA) is one of the most common human systemic autoimmune diseases affecting approximately 1% of the world's population. Although the precise etiology of RA is unknown, genetic and environmental factors seem to be involved in its pathogenesis [1]. We observed an association of tumor necrosis factor (TNF)-$\alpha$ microsatellites and the single nucleotide polymorphisms of mannose binding lectin (MBL2) with susceptibility and progression of RA in Indian population [2,3]. The frequency of the B variant (codon 54) of the MBL2 gene was observed to be lower in RA patients in comparison to the controls ($P = 6.35 \times 10^{-6}$). However, no statistically significant difference of serum MBL was observed between the RA patients and the controls [3].

RA is known to be associated with the presence of a number of autoantibodies [4,5]. Rheumatoid factor (RF) is a well known autoantibody directed against the Fc part of human $IgG_0$ (agalactosyl IgG with an exposed *N*-acetyl glucosamine), which is more prevalent in RA [6]. This agalactosylated IgG with the exposed GlcNAc is also shown to be an easy target to the serum acute phase protein, mannose-binding lectin (MBL) [7].

* Corresponding author. Tel.: +91 11 2766 2581; fax: +91 11 2766 7471.
*E-mail addresses:* hdas@igib.res.in, hasidas@yahoo.com (H.R. Das).

0896-8411/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jaut.2006.07.002

Serum MBL is enhanced by inflammatory stimuli and binds carbohydrates on the surfaces of pathogenic microorganisms and particulate materials via its C-terminal domain in a calcium dependent manner and activates lectin complement pathway in an antibody-independent manner [7,8]. MBL consists of trimeric subunits with collagenous domain that assemble to high order structures (hexamers) resembling the complement component C1q. On binding to the specific carbohydrate, its associated serine proteases (MBL associated serine proteases or MASPs) get activated, leading to activation of lectin complement pathway [6,7].

Consequently MBL has a significant role in eliciting the inflammatory response and thus has been well associated with the pathogenesis of various infectious and autoimmune diseases [9–11]. Particularly in rheumatoid arthritis (RA), an exposed GlcNAc on $IgG_0$ being an easy target for binding with MBL leads to generation of inflammatory response. The presence of MBL and $IgG_0$ in the synovial fluid of these patients supports such association [12].

MBL deficiency has been found in the general population and mostly correlated with the mutations in MBL gene. Its gene maps to chromosome 10 and mutations in the MBL gene and promoter polymorphisms determine the protein levels [3]. The variant alleles and altered serum MBL level are frequently associated with RA [13–15].

Recently in systemic lupus erythematosus (SLE), a related autoimmune disorder, autoantibodies against C1q and MBL have been reported and interestingly, the anti-MBL autoantibody level in this disorder has been shown to decrease the functional activity of MBL [16,17]. These reports prompted us to investigate the presence of MBL autoantibodies in the sera and synovial fluid of RA patients. The significant presence of the anti-MBL autoantibodies in RA directed us to assess their functional importance. The clinical significance of these autoantibodies was further measured against the conventionally known isotypes of rheumatoid factor (IgM RF and IgG RF) in RA.

## 2. Patients and methods

### 2.1. Patients and controls

Patients visiting the Rheumatology outpatient center of the Department of Rheumatology, Army Hospital, Research and Referral, N. Delhi, India were considered for the present study. All patients fulfilled the American College of Rheumatology classification criteria for the disease [18] that finally served as the gold standard for the diagnosis of rheumatoid arthritis. Blood samples from 107 RA patients were collected. To analyze the sensitivity and specificity of the tests, our control cohort of 121 individuals included healthy controls and 13 individuals with degenerative and other inflammatory joint diseases including psoriatic arthritis ($n = 3$), osteoarthritis ($n = 7$) and spondylarthropathy ($n = 3$).

All controls and the RA patients included in this study were age, sex and ethnicity matched. Informed consent was obtained from each patient and normal individual. The human ethics committee approved the project.

Medical records of the patients stating the disease duration, duration of morning stiffness, acute phase response as erythrocytes sedimentation rate (ESR) and C-reactive protein (CRP), presence of extra-articular manifestations and presence of bone deformities were collected (Table 1). Disease activity of the patients with RA was assessed at their first visit, using the DAS-28 disease score calculator, according to number of tender joints involved, swollen joints, ESR (mm/1st h), visual analogue score (VAS) for general health as subjectively estimated by the patients and clinician's assessment of physical function [19]. The RA patients ($n = 107$) were later categorized into severe ($n = 75$) and less severe ($n = 32$) groups on the basis of DAS-28 disease score calculator as well as other medical records stating the disease duration, duration of morning stiffness, presence of extra-articular manifestations, the presence of bone deformities, and the titer of CRP (as in Table 1 of this paper).

The examiner was blinded to the anti-MBL results at the time of diagnosis.

Blood samples from controls and patients were obtained at first clinical presentation and sera separated were stored at −20 °C until assayed for anti-MBL autoantibody.

Synovial fluid (SF) samples were obtained from actively inflamed joints of 23 patients with RA. Disease duration in these RA patients was $5 \pm 3.2$ years, as measured from the first clinical signs of arthritis, irrespective of which joint was initially affected. Clinical inflammation was defined as both joint swelling and pain at the time of physical examination. The laboratory assessment of these RA patients included disease duration, duration of morning stiffness, ESR, CRP, presence of extra-articular manifestations, presence of bone deformities and rheumatoid factor (RF). The samples were ejected out by the rheumatologists under aseptic conditions and were immediately stored at -70 °C until assayed. No prior freeze–thawing was allowed.

Table 1
Characteristics of 107 patients with rheumatoid arthritis (RA)

| Clinical and paraclinical variables | RA patients Mean (SD) | Severe patients Mean (SD) | Less severe patients Mean (SD) |
|---|---|---|---|
| Females (%) | 60 | 50 | 50 |
| Age (years) | 45 (6) | 43.5 (3.6) | 41 (4.6) |
| Disease duration (years) | 3 (2.8) | 3.4 (1.5) | 2 (1.5) |
| DAS | 5.1 (1.61) | ≥5.1 | <5.1 |
| Tender joint count | 10 (6) | 14.6 (3.8) | 6.2 (2.4) |
| Swollen joint count | 8 (3) | 10.3 (2.1) | 5.6 (4.2) |
| ESR (mm/1st h) | 25.8 (14.2) | 30.2 (10.5) | 26.4 (7.6) |
| CRP (mg/L) | 12 (7) | 15.5 (4.2) | 12 (7.3) |
| Morning stiffness (min) | 80.6 (69.5) | 98.7 (73.8) | 28.7 (23.2) |
| RA patients with nodules (%) | 10 | 100 | 0 |
| Anti-MBL positive (%) | 60.74 | 65.34 | 50 |
| IgM RF positive (%) | 51.4 | 56 | 40.62 |
| IgG RF positive (%) | 28.97 | 36 | 12.5 |

DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor.