ORIGINAL ARTICLES

Occurrence of nucleic acid hydrolyzing IgM antibodies in rheumatoid arthritis patients' sera

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Abstract

Objective: The study goal was to investigate the nuclease like catalytic function of polyclonal IgM antibodies (Abs) recovered from rheumatoid arthritis (RA) patient sera.

Methods: Intact polyclonal IgM Abs were recovered from the sera, in a single step, using CIM® EDA monolith column. The DNase activity and RNA hydrolysis assays, *viz. in-situ* and thermal inactivation, were performed to achieve the objectives.

Results: The RA IgM Abs exhibited moderate hydrolysis of plasmid DNA, pUC18 and total yeast RNA, respectively. Non-negligible differences in the nuclease activities were prominent among the patient's samples, in spite of an overall moderate activity. Result of RNA *in-situ* assay revealed that the catalysis function is mediated by µ chain, thereby confirming it to be an intrinsic property of IgM Abs.

Conclusion: The current preliminary study has concluded that IgM Abs from RA disease possess nuclease activity and IgMs mediate hydrolysis of both DNA and RNA macromolecules.

Keywords: Rheumatoid arthritis, IgM antibodies, catalytic antibodies, DNA hydrolysis, RNA hydrolysis

Introduction

One of the prominent features of autoimmune diseases is the presence of auto-reactive antibodies or autoantibodies (AAbs) in the patients' blood. A few of these antibodies (Abs), specifically IgG class, are well-known to possess protease and nuclease-like enzymatic functions. Such Abs are designated as natural catalytic antibodies (CatAbs).[1] Enormous studies have divulged with evidence that the catalysis phenomenon is an intrinsic function of such AAbs and they are known to efficiently hydrolyze biomolecules under *in-vitro* conditions.[2] Many research works have corroborated the presence of catalytic framework in the CatAbs and have also revealed that it almost mimics the natural enzymes. These AAbs exhibit amidase and nuclease like functions, but with a kinetic characteristic in the range of µM-mM, unlike their counterpart enzymes.[3]

Rheumatoid factor (RF) is a hallmark AAb of rheumatoid arthritis (RA) and used as a clinical marker.[4] It is an immune complex, wherein the abnormal IgM and/or IgG Abs complexes with autologous Abs. Basically, the IgM or IgG Abs interacts with the Fc region of normal autologous IgG Abs, preferably close to the carbohydrate domain.[5, 6] As a consequence, RFs are found as immune complexes in synovial fluid and in blood of RA patients, and may contribute to disease manifestation.[7] Hence, clinically RA is said to be a hyperimmunoglobulin disease condition. Many studies have meticulously demonstrated that IgG AAbs from diverse autoimmune diseases mediate catalysis of different biomolecules. However, very few studies have reported the presence of such catalytic functions in IgM Abs recovered form autoimmune diseases.[8,9,10] A previous study by our group unraveled the presence of serine protease like activity by IgM Abs from RA sera and also decoded the presence of atypical catalytic framework in IgM Abs.[11]

Besides, the IgG AAbs are also known to hydrolyze both DNA and RNA molecules.[12,13] But such a function in IgM Abs remains elusive. The present study analyzed the

plausibility of the DNA and RNA hydrolyzing function of the polyclonal IgM Abs recovered from RA patients' sera. Results indicate that the IgM Abs mediate hydrolysis of nucleic acid substrates. Subsequently, a semi-quantitative analysis was performed to comprehend the functional variances exhibited by IgM Abs among the patients.

Materials and Methods

Patient sera

The RA serum (about 0.2 ml) samples of 20 cases, who fulfilled the American College of Rheumatology (ACR) criteria, were obtained from the biobank of Groupe hospitalier Cochin-Saint, Paris, France. Healthy serum (n=5) was also obtained from the biobank.

IgM purification

Recovery of IgM Abs is explained in detail elsewhere.[11] In brief, purification of IgM Abs was carried out using CIM® Ethylenediamine (EDA) monolith column (BIA separation, Slovenia). About 50 µl of total serum, diluted twice in phosphate buffer pH 7.2, was injected into a disk type (dimension 12 X 3 mm; 0.34ml column volume) column. The unbound proteins were washed out with phosphate buffer, while the bound proteins were eluted using phosphate buffer pH 7.2 with 1M NaCl, in a linear gradient mode. Each chromatographic run was completed in 2.5 min.

DNA hydrolysis assay

Hundred nanograms of supercoiled (>80%) plasmid DNA, pUC18 (Genei, India) and 2 µg of IgM Abs recovered from each RA patient's serum were prepared in a 20µl reaction buffer comprising 20mM Tris-HCl, pH 7.5, with 1mM ethylenediamine tetra acetate (EDTA) and 5mM $MgCl_2$. DNA alone and DNA with healthy donor IgM Abs in the reaction buffer were respectively used as controls. The reaction mixture was incubated at 37°C for 2.5h. The digested end products were resolved on an agarose gel (0.8%) and were visualized by staining with ethidium bromide. The image was captured by Image Lab software, Bio Rad.

RNA hydrolysis assays

RNA *in-situ* gel

The RNA *in-situ* assay was performed as described earlier.[14] Total yeast RNA (Sigma, MO, USA) at a 200 µg/ml concentration was added to the 10% resolving SDS-polyacrylamide gel, prior to polymerization. About 3 µg of IgM Abs, recovered from the RA sera, were treated at 37°C in 1% SDS sample buffer with DTT (reducing agent) for 20 min and subsequently subjected to electrophoresis. After electrophoresis, the gel was rinsed in 20mM Tris-HCl buffer, pH 7.5 with 1mM EDTA and 15% isopropanol for 1h to remove SDS, followed by two wash in the same buffer but without isopropanol, for 30 min. Next, the gel was incubated at 37°C in 20mM Tris-HCl buffer, pH 7.5 with 1mM EDTA and 5mM $MgCl_2$ for 16h. This condition allows protein to re-nature as well enhance the hydrolysis of RNA that was in close proximity to Abs present inside the gel. Initially, the gel was stained with ethidium bromide and was visualized by a gel doc to observe RNA hydrolysis. The gel was documented by Image Lab software, BioRad. Later, the same gel was thoroughly washed by deionized water to remove ethidium bromide and followed by Coomassie blue staining to visualize the protein bands. Under similar experimental conditions, healthy donor IgM Abs and bovine pancreatic RNase A were used as controls, respectively.

Thermal inactivation assay

The reaction mixture comprising 2µg/ml of IgM Abs and 0.4 mg/ml of total yeast RNA (Sigma, MO, USA) were taken in 50mM Tris-HCl buffer, pH 8.5 with 1mM EDTA and 5mM $MgCl_2$. The reaction mixture was incubated at 37°C for 1h. Aliquots of 20µL were collected at every 10 min interval and were treated with 10% trichloroacetic acid (TCA) to attenuate the reaction. The tubes were centrifuged at 10000X g for 10 min at 4°C. The supernatant containing acid soluble oligonucleotides, liberated after RNA hydrolysis, was measured at 260nm using UV-Vis spectrophotometer (Beckman Coulter, USA). Under similar conditions, healthy donor IgM Abs and bovine pancreatic RNase A were also studied.

Results

The IgM Abs recovery from both RA patients' and controls' sera were a homogenous preparation, as described in our earlier report.[11] Current experimental data revealed that the IgM Abs mediate catalysis of the substrate, plasmid DNA, pUC18. Observation (Fig 1) of concomitant decrease in the supercoiled (SC) form of DNA and with a relative increase or decrease of the linear form (LC) of DNA clearly demonstrated that the RA IgM Abs has cleaved the DNA. For simplicity, data of few RA samples, healthy controls and native DNA are represented. While these functional characteristics were mainly noted in the RA IgM Abs, as expected; negligible activity in healthy controls and no activity in DNA without Abs (Fig. 1, lane U) were observed. Further to gain a better insight, the ratio of SC and LC DNA percent, after time bound hydrolysis, was determined by densitometry analysis

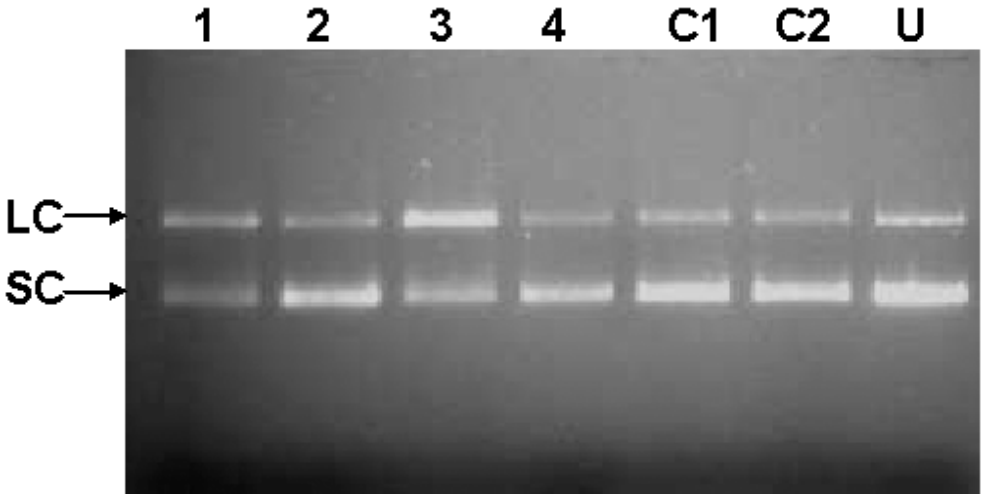
(Table I). Apparently, the IgMs from P1, P3 and P4 relatively cleaved the DNA in an efficient manner, while P2 cleavage rate was low. Despite low activity, the P2 IgM hydrolyzed the liner DNA, unlike other samples, which preferred supercoiled DNA.

Here, the RNase activity was examined by two experimental approaches to determine and emphasize that the activity is explicitly contributed by IgM Abs. A typical RNA *in-situ* SDS-PAGE, under reduced conditions, is shown in figure 2. A clear single band visualized around 70KDa (Fig. 2, lane P) in the RNA bound ethidium bromide gel depicted the hydrolysis of RNA by Abs. This band concurred with the heavy chain (μ) of IgM, as displayed by Coomassie blue staining (Fig. 2, lane P') of the same gel. On contrary, the healthy donor IgM Abs (Fig. 2, lane C) did not show activity, while the positive control bovine pancreatic RNase A (14KDa) exhibited activity. Furthermore, thermal denaturation assay strengthened the RNase like activity exhibited by IgM Abs. A comparative analysis between RA IgM (P1 & P2), control IgM (H) and RNase A (R) is displayed in figure 3. From the results, it is evident that RA IgMs progressively losses its RNA hydrolyzing function close to 30 min. But the enzyme, RNase A was functional up to 1h, while no activity was displayed by control IgM Abs. Overall, the results of RNase assays demonstrated the presence of intrinsic RNase like function in RA IgMs.

Discussion

While most of natural CatAb studies are confined to IgG Abs, the current study describes catalysis function of IgM Abs. This preliminary case-control study exemplifies the presence of nuclease-like function of IgM Abs isolate from RA patients. Despite anticipated results, observation of moderate DNA hydrolysis function by RA IgMs remains unclear. Possible reason can be structural-functional properties of IgM such as pentavalence, steric hindrance or high avidity. Intriguing variations in the activities (Table 1) can be due to functional capacities and molecular features of individual patient's IgM Abs. This argument tunes with the study of Gabibov *et al*.,

Fig. 1: DNA hydrolysis activity of IgM Abs



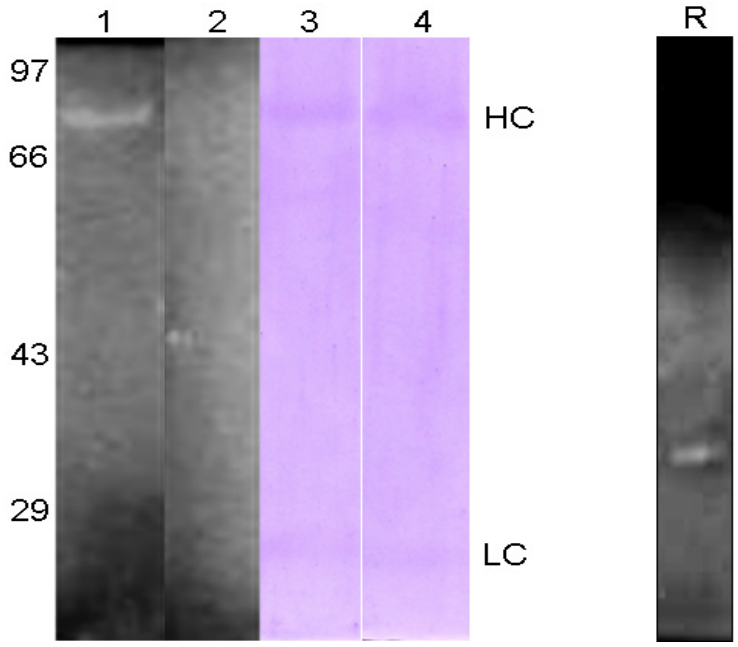
Agarose gel (0.8%) reveals degradation of super coiled (SC) DNA to linear form (L). Purified IgM Abs (2 μg) was incubated with the substrate plasmid DNA in the reaction buffer for 2½ h. Lanes: 1- 4, RA patients; C1 & C2 – Healthy donors; U- DNA alone incubated in the reaction buffer.

Table 1: Densitometry analysis of DNA hydrolysis

DNA forms	P1	P2	P3	P4	C1	C2	Intact DNA
Linear form [LC] (%)	56.6	29.0	58.6	33.9	32.2	32.1	10.1
Supercoil form [S6] (%)	43.3	70.9	41.3	66.0	67.7	67.8	89.8

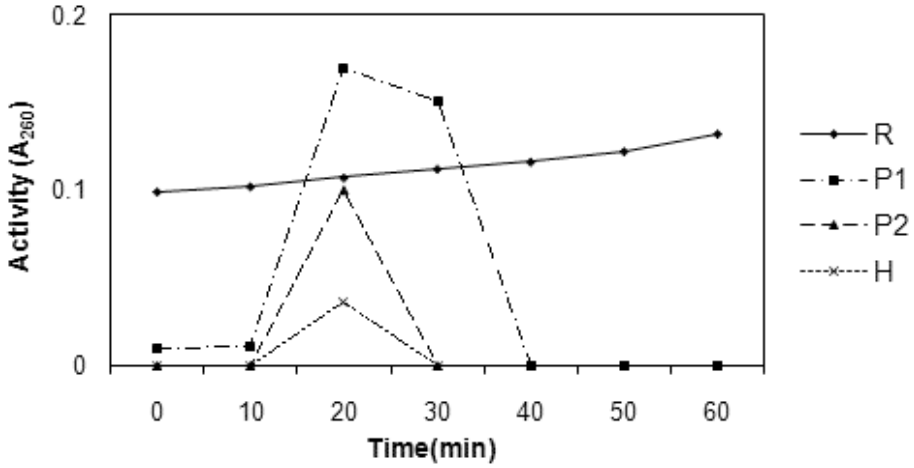
Measure of ratio of supercoiled and liner DNA forms after hydrolysis in percentage.

Fig. 2: RNA *in-situ* gel under reducing conditions



Hydrolysis of yeast total RNA by IgM Abs purified from RA patient and healthy control. Clear zone indicates the RNA hydrolysis and Coomassie staining of the same gel reveals the protein bands which superimpose the active zone. Lanes: 1 & 3, RA patient IgM; 2 & 4, healthy control IgM; R-bovine pancreatic RNase A. H-Heavy chain (70KDa) and L-Light chain (25KDa).

Fig. 3: Comparative thermal inhibition assay



IgM Abs purified from RA sera and healthy donor was incubated with the substrate yeast total RNA. Activity exhibited by RA patients, healthy donor and a positive control bovine pancreatic RNase A is shown. R- Bovine Pancreatic RNase A (♦); H - Healthy donor IgM (×); P1 (■) & P2 (▲) – IgM from different RA serum.

wherein the authors have reported such effects in the IgG Abs from RA patients.[15]

One of the challenges associated with natural CatAbs studies is that the Abs should be devoid of contamination, especially enzymes proteases or nuclease. In an effort to understand the RNase like function by IgMs and also to differentiate it from human RNase, the current study employed RNA *in-situ* SDS-PAGE and thermal denaturation approaches.[14] The former assay condition facilitates disassociation of non-covalent interaction of IgM and trace amounts of RNase that might had stemmed from serum or external sources. Further, electrophoresis resolves the proteins based on their molecular masses and display activity, if any, accordingly

(Fig. 2). The physical property, i.e. Abs are thermolabile, while RNases are thermostable, was exploited in the latter assay condition (Fig.3).[16] Loss in activity around 30 min in IgMs suggests that the IgM preparation was contaminant free. Interestingly, the RNA *in-situ* revealed that nuclease like activity in RA IgM is contributed by the heavy (μ) chain and this was further strengthened by the thermal inactivation conditions. In-lieu to this, finding by Vermeer and Norde has concluded that the Abs fragments Fab and Fc are sensitive to heat treatment, even at optimal temperature, and decreasing pH, respectively.[17] Results of these experiments not only demonstrated the presence of activity by RA IgMs but also precluded the presence of RNase contamination. Thereby, the preliminary results evidently show that RA IgM Abs possess intrinsic nuclease-like activity. A further in-depth analysis is required to dedicate nucleic acid catalysis and also needs to be examined in a significant number of cases.

Conclusion

Despite small sample size, this preliminary study demonstrates presence of both DNA and RNA hydrolyzing function in the same preparation of RA IgMs. Occurrence of intrinsic nuclease like activity particularly in μ chain of the RA IgM Abs is intriguing. But questions such as biological significance of this Ab, relevance with RA disease and pathophysiological role remain elusive.

Conflict of interest

The authors declare that they have no conflict of interest.

Citation

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