Journal of Cellular Biochemistry 40:43–47 (1989)
Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity 49–53

Antibodies to hnRNP Core Protein A1 in Connective Tissue Diseases

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We investigated the specificity of circulating autoantibodies to a heterogeneous nuclear ribonuclear protein A1 (hnRNP A1), obtained by recombinant DNA technique, in different rheumatic diseases: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), scleroderma, primary Sjogren's syndrome (SS), idiopathic Raynaud (IR), mixed connective tissue disease (MCTD), and healthy donors. All sera were tested by ELISA on hnRNP A1 protein. Positive values were obtained in 22% SLE, 19% scleroderma, 10% IR, 40% (2/5) MCTD, 5% SS, and 50% RA patients. The majority of patients reacted with the aminoterminal part (UP1) of hnRNP A1; however, some RA patients reacted also with the carboxy-terminal part that shows partial homology with keratin. Therefore, hnRNP A1 (UP1) can be considered a target of antinuclear autoimmunity in various rheumatic disorders.

Key words: systemic lupus erythematosus, rheumatoid arthritis, autoantibodies to hnRNP A1, recombinant protein, immunoblotting

Autoantibodies to various nuclear proteins are frequently found in sera of patients affected by systemic rheumatic diseases [1]. Several authors have described autoantibodies to small nuclear ribonuclear proteins (snRNP) (for example, [2]). On the contrary, only a few articles have reported the presence of autoantibodies to hnRNP; in particular, Fritzler et al. described autoantibodies that react with hnRNP particles in the chromatin of interphase cells [3]. Furthermore, Arad-Dann et al. described autoantibodies directed to 200 S nuclear RNP particles (which contain hnRNA, RNP, and snRNP) in sera of patients affected by SLE and myositis [4]. hnRNP proteins are family of a large number of polypeptides; 80% of them (called hnRNP core proteins) consist of about nine different, but related, polypeptides of 34–43 Kd as estimated from sodium dodecyl sulfate/polyacrylamide gel electrophoresis

Received May 25, 1988; accepted November 23, 1988.

(SDS-PAGE). hnRNP core proteins have been suggested to be involved (as the snRNP) in the splicing process [5,6].

We investigated the specificity of circulating autoantibodies to a hnRNP core protein (hnRNP A1), obtained from *E. coli* by recombinant DNA technique, in sera of patients affected with various systemic rheumatic diseases. hnRNP A1 is the main constituent of the hnRNP core proteins; as recently revealed by cDNA cloning [7,8], this protein has a molecular weight of 34 Kd and consists of two distinct parts: the amino-terminal part (24 Kd), which corresponds to the already known single-stranded DNA binding protein (ssDBP) termed UP1 [9] and the carboxy–terminal portion, which is very rich in glycine and shows a partial homology with human and mouse epidermal keratin [7,9].

MATERIALS AND METHODS

Patients

Sera from 31 systemic lupus erythematosus (SLE), 44 rheumatoid arthritis (RA), 26 scleroderma (PSS), 20 primary Sjogren's syndrome (SS), ten idiopathic Raynaud (IR), five mixed connective tissue diseases (MCTD), 15 inpatients suffering from various cronic cardiovascular and pulmonary diseases, and 25 healthy donors were tested by ELISA on hnRNP A1 protein.

Preparation and Purification of hnRNP A1 Protein

hnRNP A1 was overexpressed and purified as unfused 34 Kd protein from *E. coli* harboring a recombinant expression vector (pRC23) carrying the A1 cDNA coding sequence. Similarly, the NH_2-terminal domain, UP1, and the COOH-terminal portions, glycine rich, were obtained by subcloning truncated A1 cDNA versions into appropriate expression vectors (derivatives of pRC23). The recombinant proteins were purified by affinity column procedures as described [8], and the final preparations were homogenous by amino acid sequencing and by SDS-PAGE.

ELISA Technique

The hnRNP A1 protein was diluted at 2 μg/ml in 50 mM carbonate buffer, pH 9.6, plated (100 μl/well), and incubated at 4°C overnight. Plates were then washed in PBS–Tween 20, incubated for 2 hr with patient sera (diluted 1:100), washed, incubated for 2 hr with peroxidase-conjugated antibodies anti-human Ig (Dakopatts, Denmark), washed, and exposed to the substrate solution as described [10]. The optical density (O.D.) was measured at 492 nm using a Titertek multiscan reader (Flow Laboratory, Opera, Italy). Values higher than mean O.D. values + 3 S.D. of normal controls, at 1:100 dilution (O.D. = 52 ± 54), were considered positive for hnRNP A1 protein (O.D. > 260).

Immunoblotting Technique

For immunoblotting, 12 μg hnRNP A1 was mixed in 62.5 mM Tris–HCl, pH 6.8, containing 5% mercaptoethanol, 2% SDS, and 0.1% bromophenol blue, heated at 100°C for 5 min, and centrifuged. The supernatant was electrophoresed on 10% SDS polyacrylamide gel at 30 mA for 4 hr. The gel was blotted onto nitrocellulose as described by Towbin et al. [11]. The nitrocellulose sheet was cut into strips, washed extensively, and processed as described [10].

TABLE I. Binding of Patient Sera to hnRNP A1

Patients	No.	Binding to hnRNP A1 protein	
		% positive cases	Mean O.D. value
SLE	31	22	647
RA	44	50	734
PSS	26	19	408
SS	20	5	353
IR	10	10	525
MCTD	5	40	704
Inpatients	15	0	182

Results are expressed as percentage of positive cases (values higher than mean O.D. values + 3 S.D.; O.D. > 260) over the total no. patients and mean O.D. values of positive cases in different patient groups.

RESULTS

The percentage of patients presenting circulating autoantibodies to hnRNP A1 in various rheumatic disorders is shown in Table I; patients affected by RA show the highest prevalence of anti-hnRNP A1 antibodies and the highest binding values. The patients whose serum gave O.D. values (in ELISA) higher than mean O.D. values + 3 S.D. of normal donors were considered positive on hnRNP A1.

All positive sera were then tested by immunoblotting on the hnRNP A1 protein to rule out the possibility that positive reactions in ELISA could be due to the presence of contaminating *E. coli* components in the antigen. Figure 1 shows the reactions of some representative sera on hnRNP A1 (34 Kd): lanes A–E and N were stained with

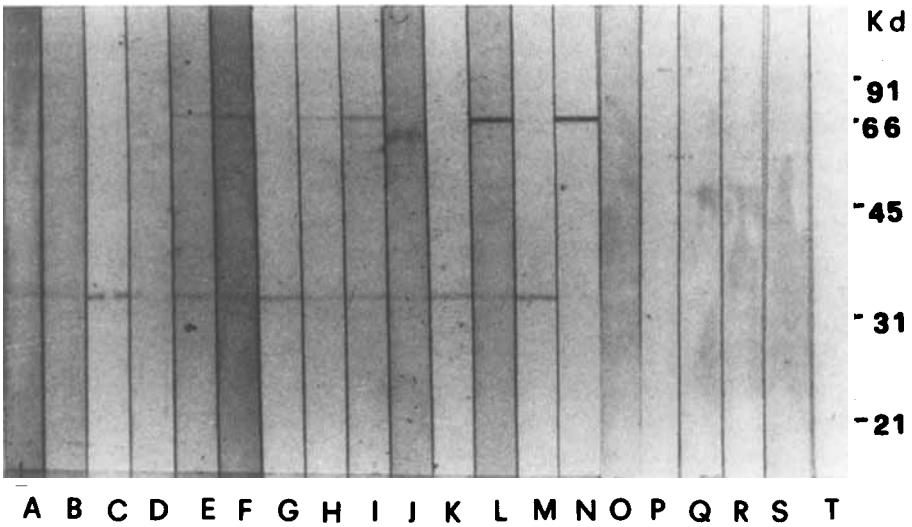

Fig. 1. Binding of different sera to hnRNP A1 by immunoblotting. Sera from patients found to be positive (**lanes A–N**) or negative (**lanes O–T**) on hnRNP A1 using ELISA were diluted 1:100 and incubated with the strips.

TABLE II. Binding to UP1 Peptide and/or Glycin-rich Part of hnRNP A1 Protein

Patients	No.	Binding to		
		UP1	UP1 + Gly	Gly
SLE	7	100	0	0
RA	22	64	18	18
PSS	5	80	20	0
Other	4	100	0	0

Results are expressed as percentage of positive cases to UP1 and/or Gly over the total number of patients presenting antibodies to hnRNP A1.

positive sera from RA patients; lanes F and I–K, with positive sera from SLE patients; lane G, with positive serum from MCTD; lanes H and L, with positive sera from PSS, and lane M, with positive serum from SS. Lanes O and P were stained with negative sera from RA patients; lanes Q and R, with negative sera from SLE patients; and lanes S and T, with sera from normal donors. Lane N was stained with serum from a patient who reacts with a contaminating protein at 70 Kd and not with hnRNP A1 (34 Kd). Such a patient was considered negative and not included among the positive cases reported on Table I.

Because hnRNP A1 consists of two parts (see Introduction), one of which is very rich in glycine and shows homology with keratin, we investigated whether the positivity of autoantibodies to hnRNP A1 could be due to the keratin homologous part of the protein (glycine rich) or to the specific part of the protein (UP1). Table II shows that all the patients except RA patients present autoantibodies only to UP1; 64% of RA patients present autoantibodies only to UP1, but 18% present autoantibodies both to UP1 and the glycine rich part of hnRNP A1 (called "gly" in Table II) and 18% present autoantibodies that react only with the glycine rich part of hnRNP A1 that shares homology with keratin (called "gly" in Table II).

DISCUSSION

The presence of autoantibodies to hnRNP have been described in patients with different connective tissue diseases [3,4], but only few data are available about the specific polypeptides involved in anti-hnRNP autoimmunity. The aim of this study is the detection of circulating antibodies to hnRNP A1 obtained by recombinant DNA technology [7,8] in various autoimmune rheumatic disorders. Our data show that hnRNP A1 can be considered a target of antinuclear autoimmunity in various rheumatic disorders. A very high prevalence of anti-hnRNP A1 antibodies has been found in rheumatoid arthritis, a disease usually considered poorly associated to specific antinuclear antibodies. The possibility that sera containing rheumatoid factors (RF) may give false-positive results is in contrast with the results obtained by immunoblotting and the finding that 19/20 patients affected with SS (80% of whom were RF positive) were negative for hnRNP A1 by ELISA (Table I).

The study of circulating autoantibodies to pure proteins (such as proteins obtained by means of recombinant DNA technology) may also disclose cross reaction with other proteins because of the partial structural homology of different molecules. For example, RA has also been reported to be associated with antikeratin antibodies

[12]. Therefore, it is possible that the autoantibodies to hnRNP A1 described here may cross react with the carboxy-terminal (glycine-rich) portion of hnRNP A1 protein that shows a partial homology in primary structure with keratin [7]. In fact, our results indicate that autoantibodies to the glycine-rich part are found almost only in sera of RA patients; in particular, 18% hnRNP A1-positive RA patients react only with the glycine-rich part, 18% have both autoantibodies to UP1 and the glycine-rich part, and 64% have antibodies only to UP1. We are now investigating if autoantibodies to keratin may play a role in anti-hnRNP activity in RA patients.

ACKNOWLEDGMENTS

This work was supported by a grant, "Progetto Finalizzato Biotecnologie e Biostrumentazioni," from Consiglio Nazionale delle Ricerche, Roma, Italy. A.C. is supported by a fellowship from "Fondazione Buzzati-Traverso," Roma, Italy.

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