Heterogeneous Nuclear Ribonucleoprotein H1, a Novel Nuclear Autoantigen

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BACKGROUND: Serum samples from patients with autoimmune connective tissue diseases that show a finely speckled antinuclear antibody (ANA) on indirect immune-fluorescence often have antibodies against unknown nuclear target antigens. To search for such autoantigens we applied a proteomic approach using sera from patients with a high ANA titer (≥640) and finely speckled fluorescence but in whom no antibodies to extractable nuclear antigens (ENA) could be identified.

METHODS: Using an immunoproteomics approach we identified heterogeneous nuclear ribonucleoprotein H1 (hnRNP H1) as a novel nuclear target of autoantibody response.

RESULTS: Recombinant rat hnRNP H1 reacted in Western blot analyses with 48% of 93 sera from patients with primary Sjögren syndrome and with 5.2% of 153 sera from patients with other connective tissue diseases (diseased controls). For comparison, the diagnostic sensitivity and specificity of anti–Sjögren syndrome A (SSA) antibodies for primary Sjögren syndrome in the same patient cohort were 88.2% and 76.3%, respectively. Interestingly, 5 of 11 primary Sjögren syndrome patients with no anti-SSA or anti-SSB antibodies had anti–hnRNP H1 antibodies. Anti–hnRNP H1 antibodies were preabsorbed by hnRNP H1, as demonstrated by indirect immunofluorescence. In an evaluation of the presence of anti–hnRNP H1 antibodies in 188 consecutive samples submitted to the clinical laboratory with positive ANA (titer ≥160), anti–hnRNP H1 antibodies were found in 3 of 7 (2 primary and 5 secondary) Sjögren syndrome patients and in 8.3% of the diseased controls.

CONCLUSIONS: HnRNP H1 is a newly discovered autoantigen that could become an additional diagnostic marker.

Sjögren syndrome is an autoimmune exocrinopathy, characterized by dryness of the mouth and eyes resulting from a chronic loss of secretory function of the salivary and lacrimal glands (1). Extraglandular systemic manifestations are common and include skin abnormalities, arthralgia, myalgia, thyroiditis, and pulmonary, renal, gastrointestinal, hematological, cardiac, and neurological abnormalities (2). The etiology of the disease remains unclear (2). The prevalence of the disease is estimated to be 0.5%, and there is a female preponderance (2). Pathological findings involve focal lymphocytic infiltration of affected tissues. Laboratory findings comprise hypergammaglobulinemia, rheumatoid factor, and autoantibodies against salivary ductal cells and Sjögren syndrome A (SSA)9 and SSB antigens. Depending on the technique used, anti-SSA antibodies are found in 45%–90% of patients with Sjögren syndrome (3). Anti-SSB antibodies are found slightly less commonly (1). Anti-SSA antibodies are not specific for Sjögren syndrome; they are found in other systemic diseases such as systemic lupus erythematosus (SLE), subacute cutaneous lupus erythematosus, and neonatal lupus (3).
Because of the nonspecific symptoms (dry eyes and mouth), establishment of the diagnosis of Sjögren syndrome may be difficult (1). There is an unmet need for additional accurate and valid diagnostic markers. A number of new autoantigens have recently been suggested: α-fodrin, muscarinic M3 acetylcholine receptor, and SS-S6 (4–6), which is structurally related to the 52-kDa SSA antigen. These markers, however, have not shown adequate specificity and sensitivity to serve as valuable clinical markers, or are not available for routine diagnostic tests (1, 7–9). The fact that there are patients with (undifferentiated) autoimmune connective tissue disease who have antinuclear antibodies (ANA) in high titers but in whom no antibodies to extractable nuclear antigens (ENAs) (e.g., SSA or SSB) can be identified (10) prompted us to search for novel autoantigens.

Materials and Methods

STUDY POPULATION

Three groups of serum samples were included in the study. Group 1 consisted of samples obtained from well-defined patients with an autoimmune connective tissue disease (n = 246). This group included patients with SLE (n = 41, male/female ratio 5:36, mean age 45 years, range 23–80 years), primary Sjögren syndrome (n = 93, male/female ratio 9:84, mean age 60 years, range 16–94 years), scleroderma (n = 39, male/female ratio 10:29, mean age 55 years, range 22–72 years), dermatomyositis (n = 15, male/female ratio 6:9, mean age 48 years, range 25–80 years), polymyositis (n = 7, male/female ratio 2:5, mean age 57 years, range 47–70 years), rheumatoid arthritis (RA) (n = 42, male/female ratio 13:29, mean age 58 years, range 33–90 years), and mixed connective tissue disease (MCTD) (n = 9, male/female ratio 2:7, mean age 45 years, range 18–63 years). Ten of these patients had secondary Sjögren syndrome (n = 10, male/female ratio 3:7, mean age 54 years, range 34–86 years); 4 in combination with SLE, 2 with scleroderma, 1 with dermatomyositis, 2 with RA, and 1 with MCTD. The samples were obtained from patients attending the University Hospitals Leuven (Belgium). Seventy-seven consecutive patients with primary Sjögren syndrome were from Rotterdam Erasmus MC, University Medical Center Rotterdam (the Netherlands). The prevalence of ANA positivity in this patient group was 65.9%.

The second group of samples consisted of 188 consecutive serum samples submitted to the clinical laboratory for ANA testing (titer ≥160). The third group included ANA-negative serum samples from healthy individuals (n = 41, male/female ratio 4:37, mean age 45 years, range 27–63 years).

All patients with primary or secondary Sjögren syndrome had disease characteristics that conformed with the American-European consensus classification criteria (11). Patients with SLE, scleroderma, and RA met the classification criteria of the American College of Rheumatology (12–14). Patients with poly- and dermatomyositis met the criteria of Bohan and Peter (15), and patients with MCTD met the criteria of Alarcón-Segovia (16). The serum samples that were used for this study were from the serum data bank. Samples were obtained from patients as part of routine screening for autoantibodies in the clinical laboratory. There was no informed consent for this study, but the study was approved by the local ethics committee.

ANA TESTING, IDENTIFICATION OF ENA ANTIBODIES, AND COUNTERIMMUNOELECTROPHORESIS

ANAs were determined by indirect immunofluorescence using HEp-2000® cells (Immunococepts) with an Axioplan 2 fluorescence microscope (Carl Zeiss Microimaging) and Cytovision 3.6 software (Applied Imaging Corporation). The presence of antibodies to ENAs was assayed by ENA dot blot (Biomedical Diagnostics), UniCap100, and/or UniCap250 system (Phadia Diagnostics), ANA3 test (anti-SSA 52/anti-SSA 60 status, Euroimmun), or counterimmunoelectrophoresis with rabbit thymus as the antigenic source (17).

PREPARATION OF RECOMBINANT HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN H1

Recombinant his-tagged heterogeneous nuclear ribonucleoprotein H1 (hnRNP H1) protein from rats was prepared as previously described (18). Recombinant GST-tagged hnRNP H1 protein from humans was prepared from cDNA (a gift from Prof. D. Black) according to Devogelaere et al. (19).

GEL ELECTROPHORESIS AND WESTERN BLOTTING

A salt-based protein extract from rabbit thymus (20 μg) or 300 ng of recombinant hnRNP H1 was separated by 1-dimensional SDS-PAGE (12.5%). For the separation of proteins in 2 dimensions, immobilized pH-gradient strips (pH range 3–10, GE Healthcare) were rehydrated overnight with approximately 300 μg of protein extract from rabbit thymus. Loaded proteins were first separated by isoelectric focusing and then by SDS-PAGE (12.5%) on a Multiphor II Electrophoresis System (GE Healthcare) according to the supplier’s instructions. The proteins were either subjected to Western blotting or stained by Coomassie Brilliant blue.

For Western blot analysis, polyvinylidene difluoride membranes with electrotransferred proteins (Hybond-P and Novablot apparatus; GE Healthcare) were consecutively treated with 5% (wt/vol) BSA, hu-
man serum (1:500, overnight), goat antihuman IgG (1:5000), and horseradish peroxidase–conjugated rabbit antibody IgG (1:5000) with intermittent washings in Tris-buffered saline (3 × 10 min). Protein–antibody interactions were visualized by use of 0.7 mmol/L 3,3′-diaminobenzidinetetrahydrochloride. Blotting signals were captured with a Typhoon 9400 scanner set on green laser light (532 nm), filter 526 SP (short pass), PMT (photo multiplier tube) 640 V, at normal sensitivity and quantified by ImageQuant TL software (both from GE Healthcare). We included a positive and negative control serum sample in each run. The CV of the positive control was 23.8% over 13 runs.

**PROTEIN IDENTIFICATION BY MALDI-TOF/TOF**

Gel pieces containing the protein of interest were washed with HPLC-grade water, dried in a Speed Vac (Savant), and digested overnight at 37 °C with 10 μL of 25 mg/L trypsin (sequence grade) in 200 mmol/L ammonium bicarbonate. The resulting peptide mixture was subjected to a ZipTip C18 cleanup and analyzed by MALDI-TOF/TOF (Applied Biosystems 4800 Proteomics Analyzer) in the presence of α-cyano 4-hydrocinamic acid (HPLC grade).

**STATISTICAL ANALYSIS**

We performed ROC analysis, Mann–Whitney analysis, and Fisher exact test by using Analyze-It for Microsoft Excel (version 2.09). Nonparametric Spearman correlation was performed with SPSS 15.0 for Windows.

**Results**

**IDENTIFICATION OF ANTI–hnRNP H1 ANTIBODIES**

In an attempt to identify new target antigens of ANA we applied an immunoproteomics approach using serum from a 43-year-old patient who had a high ANA titer (>1280) but with no reactivity to SSA, SSB, U1-RNP, Sm, Scl-70, or Jo-1. Counterimmunoelectrophoresis showed a positive precipitation line. Laboratory investigations revealed a polyclonal gammopathy and a positive rheumatoid factor. The patient’s diagnosis was determined to be undifferentiated autoimmune connective tissue disease. Primary Sjögren syndrome was suspected but the patient’s disease characteristics did not fulfill the European Union–US classification criteria (11).

Sera from the patient and from a control individual were used for Western blotting after 2-dimensional gel separation of rabbit thymus extract. Proteins to which there was reactivity were excised and identified by MALDI-TOF/TOF analysis. Alpha-enolase reacted with serum from the patient and from the control, whereas β-actin, heat shock protein 70 kDa, hnRNP A2/B1, and hnRNP H1 reacted only with serum from the patient. In subsequent analyses we focused on hnRNP H1 because this is a common nuclear matrix protein (20) that has not previously been described as autoantigen in human disease. We confirmed the presence of anti–hnRNP H1 antibodies in the patient’s serum by 1-dimensional SDS-PAGE and Western blotting using recombinant hnRNP H1 from rat and human. In both cases, a positive blotting signal was obtained with serum from the patient but not from the control (Fig. 1 A, B). HnRNP H1 from rat (Q8VHV7|Q8VHV7_RAT, Swiss-Prot Database) has 99% similarity with human hnRNP H1 (P31943|HNRH1_HUMAN, Swiss-Prot Database) but lacks 77 N-terminal amino acids.

We confirmed that anti–hnRNP H1 antibodies give a speckled nuclear fluorescence pattern on indirect immunofluorescence analysis. First, incubating HEp-2 cells with a commercial rabbit anti–hnRNP H1 antibody resulted in a finely speckled ANA pattern (Fig. 2 A). Second, preabsorption of a serum sample with reactivity to hnRNP H1 with increasing concentrations of recombinant rat hnRNP H1 resulted in a gradual decrease of the finely speckled fluorescent signal on indirect immunofluorescence analysis (Figs. 2 B-E).

Preabsorption of a serum sample with reactivity to hnRNP H1 with recombinant rat hnRNP H1 also resulted in decreased reactivity on Western blotting (data not shown).

**ANTI–hnRNP H1 ANTIBODIES IN PATIENTS WITH CONNECTIVE TISSUE DISEASE**

To examine whether anti–hnRNP H1 antibodies are associated with Sjögren syndrome, serum samples from a cohort of 246 patients with well-defined autoimmune connective tissue disorders were tested for reactivity to hnRNP H1 in Western blot analysis. The results are shown in Fig. 1 B and Fig. 3. We performed Mann–Whitney analysis to test for the prevalence of anti–hnRNP H1 antibodies in patients with primary Sjögren syndrome vs SLE, scleroderma, MCTD, polymyositis, dermatomyositis, and RA and in healthy controls. Anti–hnRNP H1 reactivity was significantly higher in patients with primary Sjögren syndrome compared to patients with other connective tissue disease ($P = 0.0045$). An ROC analysis was performed for anti–hnRNP H1 antibodies as a marker of primary Sjögren syndrome. The area under the curve was 0.83 ($n = 93$ patients with primary Sjögren syndrome vs 153 diseased controls). The diagnostic sensitivity of anti–hnRNP H1 antibodies for primary Sjögren syndrome was 48.4% when a cutoff (peak × area 1.90) was used that corresponded to a specificity of 94.8% in dis-
eased controls. When a cutoff (peak \times area 0.71) was used, which maximized the sum of sensitivity and specificity, the diagnostic sensitivity of anti–hnRNP H1 antibodies for primary Sjögren syndrome was 74.2% and the specificity 81.7% \textit{(Youden index 0.559 (21))}. In all subsequent analyses, the cutoff based on a specificity of 95% was used. Anti–hnRNP H1 antibodies were found in 48% (45 of 93) patients with primary Sjögren syndrome, 2.4% (1 of 41) patients with SLE, 22% (2 of 9) patients with MCTD, 7.7% (3 of 39) patients with scleroderma, 0% (0 of 15) patients with dermatomyositis, 0% (0 of 7) patients with polymyositis, 4.8% (2 of 42) patients with RA, and 5% (2 of 41) healthy individuals. Fisher exact test indicated a statistically significant difference (\( P = 0.025 \)) between the prevalence of anti–hnRNP H1 antibodies in patients with primary Sjögren disease and the prevalence in healthy individuals and in patients with other connective tissue diseases [i.e., primary Sjögren syndrome (SSp) (\( n = 6 \))], SLE (\( n = 3 \)), MCTD (\( n = 2 \)), scleroderma (SSc) (\( n = 3 \)), dermatomyositis (DM) (\( n = 2 \)), polymyositis (PM) (\( n = 2 \)), and RA (\( n = 3 \))] using recombinant hnRNP H1 from human (H) and rat (R). Molecular weight marker is indicated by M.

**Fig. 1. Reactivity to hnRNP H1 on Western blot analysis.**

(A) shows on the left side recombinant hnRNP H1 from human (H), molecular weight (MW) 76 kDa [49 kDa + glutathione S-transferase (GST)-tag], and from rat (R) (MW weight 41 kDa) separated on SDS-PAGE and stained with Coomassie Brilliant Blue. GST-tag of 27 kDa is indicated by an arrow. The right side shows recombinant hnRNP H1 from human (H) and rat (R), subjected to SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. The membrane was incubated with purified anti–hnRNP H1 antibody. (B) shows Western blot analysis of representative serum samples of a healthy control person and of patients with autoimmune connective tissue diseases [i.e., primary Sjögren syndrome (SSp) (\( n = 6 \)), SLE (\( n = 3 \)), MCTD (\( n = 2 \)), scleroderma (SSc) (\( n = 3 \)), dermatomyositis (DM) (\( n = 2 \)), polymyositis (PM) (\( n = 2 \)), and RA (\( n = 3 \))] using recombinant hnRNP H1 from human (H) and rat (R). Molecular weight marker is indicated by M.
ANTI–hnRNP H1 ANTIBODIES IN A ROUTINE CLINICAL LABORATORY SETTING

We subsequently investigated the presence of anti–hnRNP H1 antibodies in samples submitted for ANA testing (n = 188). This approach may give information on the repertoire of diseases that may lead to the production of anti–hnRNP H1 antibodies. Anti–hnRNP H1 antibodies were identified in 18 of 188 consecutively obtained ANA-positive (titer ≥160) samples. The fluorescence patterns in these 18 samples were speckled (n = 4), homogenous (n = 13), and nuclear dots (n = 1). Anti–hnRNP H1 antibodies were present in 3 of 7 patients with Sjögren disease (primary or secondary). None of the 18 patients had primary Sjögren diseases, whereas 3 of the 18 patients had secondary Sjögren syndrome, 1 in combination with SLE and 2 in combination with RA [treated with anti–tumor necrosis factor α (TNF-α)]. The patient with SLE had anti–SSA and anti–SSB antibodies. One of the patients with RA and secondary Sjögren syndrome had anti–SSA antibodies, whereas the other patient had no anti–SSA antibodies or anti–SSB antibodies. One patient had sicca complaints, Raynaud phenomenon, hypergammaglobulinemia, and cryoglobulinemia. Primary Sjögren syndrome was assumed in this patient, but he didn’t fulfill the EU-US classification criteria (11). Another patient had undifferentiated connective tissue disease with polyarthritis and autoimmune thyroid disease. Other pathologies included SLE (n = 2), ankylosing spondylitis (n = 2, both receiving anti-
TNF-α), MCTD (n = 2), and 1 each with RA (receiving anti-TNF-α), idiopathic thrombocytopenic purpura, Wegener granulomatosis with idiopathic thrombocytopenic purpura, polyarticular juvenile idiopathic arthritis, ulcerative colitis and spondylarthropathy, liver transplantation, and no diagnosis. An overview of the clinical conditions in the 188 patients as well as the anti–hnRNP H1 and the anti-SSA antibody status is provided in Table 2. In this patient group, the diagnostic sensitivity and specificity of anti–hnRNP H1 for Sjögren syndrome were 42.9% and 91.7%, respectively. For anti-SSA antibodies, the diagnostic sensitivity and specificity were, respectively, 71.4% and 89.0%. The likelihood ratios were 5.16, 0.62, 6.46, and 0.32 for,

![Fig. 3. Anti–hnRNP H1 antibodies in patients with autoimmune connective tissue diseases: SSp (primary Sjögren syndrome), SLE, MCTD, SSc (scleroderma), DM (dermatomyositis), PM (polymyositis), and RA.](image-url)

For serum samples obtained from patients with various autoimmune connective tissue diseases, reactivity to hnRNP H1 was determined by Western blot analysis using recombinant hnRNP H1 from rat. The figure shows the peak × area value (divided by 106) as measured by densitometry for several patient groups: For each group, the median peak × area value is indicated. *Statistically significant difference from all other groups (Mann–Whitney).

![Table 1. Anti–hnRNP H1 antibodies as a function of anti-SSA (52 and 60 kDa) and anti-SSB antibodies in patients with primary Sjögren syndrome (n = 93). Focus score of the anti–hnRNP H1–positive serum samples is indicated.](image-url)

<table>
<thead>
<tr>
<th>Sera (n)</th>
<th>Anti-SSA (52 and 60) antibody status</th>
<th>Anti-SSB antibody status</th>
<th>Anti–hnRNP H1 positive, n</th>
<th>Focus score of anti–hnRNP H1 positive samples</th>
<th>hnRNP H1–positive sera, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>+</td>
<td>+</td>
<td>29</td>
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<td></td>
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<td>5 ND*</td>
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<td>28</td>
<td>+</td>
<td>–</td>
<td>11</td>
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<td>–</td>
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<td>5 &gt; 1</td>
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* ND, not determined.
Anti–hnRNP H1 antibodies are an interesting diagnostic marker in anti-SSA and/or anti-SSB seronegative patients with primary Sjögren syndrome. We found that 5 of 11 (45%) of anti-SSA/anti-SSB seronegative patients had anti–hnRNP H1 antibodies. Moreover, anti–hnRNP H1 positivity may help to differentiate between patients with primary Sjögren syndrome and SLE (potentially with secondary Sjögren syndrome) among anti-SSA— or anti-SSB—positive patients, because the prevalence of these autoantibodies in SLE is low. In contrast, the differentiation between patients with primary Sjögren syndrome and MCTD is difficult (based on results on a very small number of patients) and should be further tested on larger numbers of patients to evaluate the diagnostic performance of the test.

Anti–hnRNP H1 antibodies were found not only in samples with a speckled ANA pattern, but also in samples with a homogeneous staining (nuclear dots) pattern. Five of the 18 patients with anti–hnRNP H1 antibodies received Infliximab, a TNF-α blocking drug, and displayed a homogeneous staining on ANA indirect immunofluorescence analysis. One of these patients had RA and secondary Sjögren syndrome and displayed anti–double-stranded DNA antibodies. It is known that Infliximab can induce the formation of autoantibodies, especially anti–double-stranded DNA antibodies, respectively, hnRNP H1 positive, hnRNP H1 negative, SSA positive, and SSA negative. It should be pointed out that anti–hnRNP H1 antibodies as well as anti-SSA antibodies are found in a wide range of medical conditions with a possible autoimmune pathogenesis [22].

### Discussion

We describe here hnRNP H1 as a newly recognized target of autoantibodies. These antibodies are mainly found in patients with primary and secondary Sjögren syndrome but are also present in patients with other autoimmune-mediated diseases.

The 43% diagnostic sensitivity of anti–hnRNP H1 antibodies for primary and secondary Sjögren syndrome was lower than the 85.3% sensitivity of anti-SSA antibodies [71.4% in the smaller group of Sjögren disease patients (n = 7) in the group of consecutive samples]. The diagnostic specificity of 91.7%—94.4% for anti–hnRNP H1 antibodies tended to be higher than the 78.6%—89% specificity of anti-SSA antibodies. The low specificity of anti-SSA antibodies is mainly related to the high prevalence of anti-SSA antibodies in patients with SLE. In contrast to anti-SSA antibodies, anti–hnRNP H1 antibodies were found in a great variety of autoimmune diseases, as revealed by the second cohort composed of 188 consecutive ANA-positive serum samples. The likelihood ratio of a positive test result was slightly higher for anti–hnRNP H1 antibodies than for anti-SSA antibodies in the first cohort (n = 246) but not in the second cohort (n = 188). The likelihood ratio of a negative test result for Sjögren disease was lower for anti-SSA antibodies than for anti–hnRNP H1 antibodies. The positive predictive value of anti–hnRNP H1 for Sjögren syndrome is comparable to the positive predictive value of anti-SSA for Sjögren syndrome, whereas the negative predictive value is higher for anti-SSA antibodies than for anti–hnRNP H1 antibodies.

### Table 2. Clinical conditions and anti–hnRNP H1 and anti-SSA antibodies in 188 consecutive samples submitted to the clinical laboratory with an antinuclear antibody titer ≥1:160.

<table>
<thead>
<tr>
<th>Medical condition</th>
<th>Anti–hnRNP H1, n</th>
<th>Anti-SSA, n</th>
<th>Total, n</th>
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<tr>
<td>Sjögren syndrome, primary</td>
<td>0</td>
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<td>2</td>
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<tr>
<td>Sjögren syndrome, secondary</td>
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<td>5</td>
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<td>18</td>
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<td>19</td>
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<td>5</td>
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<tr>
<td>Dermatomyositis</td>
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<td>1</td>
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<tr>
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<td>7</td>
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<td>0</td>
<td>1</td>
</tr>
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<tr>
<td>Total</td>
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<td>25</td>
<td>188</td>
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*Miscellaneous: cervicobrachialgia (n = 1), chronic urticaria (n = 1), chronic fatigue syndrome (n = 7), degenerative disease (n = 3), diabetes (n = 1), fibromyalgia (n = 1), no diagnosis (n = 9), Goodpasture (n = 1), thrombosis (n = 1), leukocytoclastic vasculitis (n = 1), obesity (n = 1), liver transplantation (n = 1), liver dysfunction (n = 1), lung fibrosis (n = 1), lymphoma (n = 1), macrophage activating syndrome (n = 1), mitochondrial myopathy (n = 1), multiple sclerosis (n = 3), nephritic syndrome (n = 1), encephalopathy (n = 1), cholecystectomy (n = 1), pityriasis lichenoides (n = 1), pneumonia (n = 4), polyarthitis (n = 1), polymyalgia rheumatica (n = 1), primary biliary cirrhosis (n = 1), psoriasis (n = 1), psoriatic spondylarthropathy (n = 1), paraparesis lower limbs (n = 1).
antibodies (23). We assumed that in such cases other antibodies masked the typical speckled pattern caused by anti–hnRNP H1 antibodies (23).

Patients with Sjögren syndrome have antibodies to SSA, SSB, and hnRNP H1. These autoantigens are all ribonucleoproteins: SSA and SSB belong to the small cytoplasmic (Y) RNPs, whereas hnRNP H1 belongs to the heterogeneous nuclear RNPs. Y RNPs are involved in nuclear export, quality control of misfolded small RNAs, and stabilization of small noncoding Y RNA (24–26).

hnRNPs comprise a group of approximately 30 different proteins (termed hnRNP A1 through U). They mainly play a role in processing of precursor mRNA, but are also involved in telomeric DNA synthesis and cellular apoptotic processes (27–29). hnRNPs are composed of at least 1 RNA recognition motif, also termed the RNA-binding domain. Some hnRNP proteins (e.g., hnRNP K and I) have been shown to associate with a subset of Y RNAs (30).

hnRNP proteins appear to be an important target of autoimmune responses. Several different hnRNP proteins (A1, A2, B, C, I, K, and R) have been described as autoantigens, as reviewed by Caporali et al. (27). Antibodies to hnRNP A1 as well as antibodies to hnRNP A2, and its alternatively spliced variants B1 and B2, (the RA33 complex) have been described in RA, SLE, and MCTD (27, 31, 32). Anti–hnRNP I antibodies seem to be associated with (pre)-systemic sclerosis (33). Antibodies to hnRNP C1/C2 have been reported, but it is not known whether they are related to a specific disease (34). Recently, hnRNP L was identified as a novel autoantigen associated with anti–hnRNP A/B antibodies (29). hnRNP D (AUF1) was described as an autoantibody target in patients with systemic rheumatic diseases (mainly SLE and RA) (35).

Members of the hnRNP H family (hnRNP H1, H2, F, and H3) are ubiquitously expressed and have been implicated in multiple aspects of mRNA biogenesis: splicing, polyadenylation, capping, export, and translation of cellular and viral mRNAs (36, 37). They have also been shown to interact with both splicing enhancers and silencers (36) and to be involved in alternative splicing and apoptosis (38–40). Many nuclear autoantigens are ribonucleoproteins and are involved in RNA and DNA processing. We identified hnRNP H1 as a novel nuclear autoantigen. Some members of the hnRNP family have previously been identified as autoantigens. Future studies that evaluate whether other members of the hnRNP family are also targets of autoimmune reactivity might reveal additional novel autoantigens. Such studies should offer new perspectives on the role of the hnRNP family in autoimmune diseases.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References


