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Antibodies to heterogeneous nuclear ribonucleoprotein H1 are directed to RNA recognition motif 3

SIR, HnRNPs are a family of ~30 proteins that associate with RNA polymerase II transcripts. HnRNPs are involved in many cellular functions, such as mRNA trafficking, splicing, telomere length control, mRNA stability, transcription and polyadenylation [1]. Most hnRNPs have at least one RNA recognition motif (RRM) (the RNA binding domain).

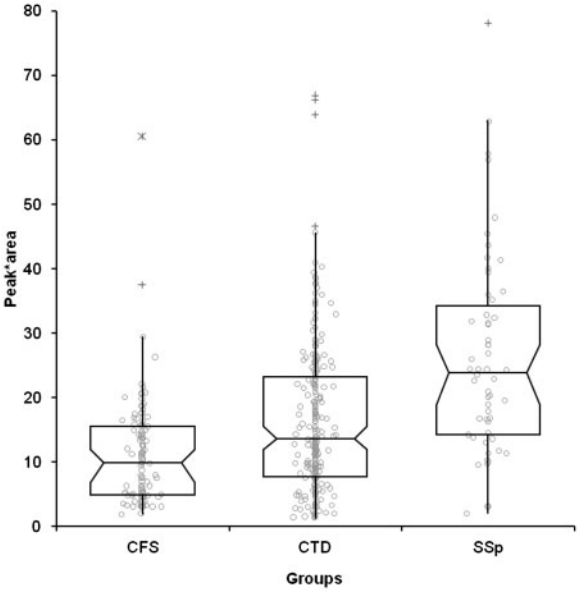
HnRNPs are increasingly recognized as targets of autoantibodies. Antibodies to hnRNP-A2 are found in patients with RA, MCTD and SLE [2]. We recently identified hnRNP-H1 as a novel autoantigen in systemic rheumatic diseases, in particular SS [3]. Anti-hnRNP-A2 antibodies are directed to an RNA binding region of hnRNP-A2 [2]. It is not known whether anti-hnRNP-H1 antibodies also recognize an RNA binding domain. In the present report, we evaluated whether anti-hnRNP-H1 antibodies are directed to a conserved RRM in hnRNP-H1.

HnRNP-H1 is a 449-amino-acid-long protein with three RRMs [4]. The first two RRMs are separated by 21 amino acids, whereas the third RRM is 100 amino acids downstream of the second RRM [4]. We prepared five fragments of human hnRNP-H1, the first fragment extended from amino acid 1 to 110 and encompasses RRM 1 (amino acids 11–90). The second fragment extended from amino acid 111 to 197 and includes RRM 2 (amino acids 111–188). The third fragment extended from amino acid 198 to 285. The fourth fragment extended from amino acid 286 to 386 and includes RRM 3 (amino acids 289–364). The fifth fragment extended from amino acid 387 to 449 (includes a glycine rich region). Recombinant his-tagged fragments of hnRNP-H1 were prepared as described previously [5]. The hnRNP-H1 fragments were amplified by means of PCR using specific primer pairs (available upon request). Cloning of the PCR products was performed using the recombination-based Gateway technology (Invitrogen Corporation, Carlsbad, CA, USA) according to the supplier's instructions. The PCR products were first ligated into the pDONR221 entry vector with a BP (*att*B, *att*P recombination sites for bacteriophage λ in *Escherichia coli*) clonase reaction and then inserted into the pDEST17 expression vector with an LR (*att*L, *att*R recombination sites for bacteriophage λ in *E. coli*) clonase reaction. Expression and purification of the recombinant protein fragments were performed as described [5].

Sera from a cohort ($n = 298$) of patients with various CTDs [DM ($n = 29$), MCTD ($n = 19$), PM ($n = 18$), SLE ($n = 71$), SSc ($n = 58$), primary SS (SSp; $n = 56$), RA ($n = 47$)] and with chronic fatigue syndrome (CFS; controls; $n = 106$) were tested for the presence of antibodies to each of the four hnRNP-H1 fragments by western blotting (for description of the method, see Ref. [5]). Patients with CFS ($n = 106$) served as controls. In these patients, all organic disease (such as CTD) was excluded. An example of western blotting analysis is available upon request. All patients with SS had disease characteristics that conformed with the American–European consensus classification criteria [6]. There was no informed consent for this study, but the study was approved by the local ethics committee (University Hospitals Leuven, Leuven, Belgium).

The reactivity to Fragments 1, 2 and 5 was not higher in patients with a CTD than in controls (data not shown). Reactivity to Fragment 4, which contains RRM 3, was higher in patients with syndromes than in controls (Kruskall–Wallis, $P < 0.0001$) or patients with other CTDs (Kruskall–Wallis, $P < 0.0001$; Fig. 1). The reactivity to Fragment 4 in patients with a CTD other than SS was higher than the reactivity found in controls (Kruskall–Wallis, $P < 0.0001$). This pattern of reactivity was very similar to the pattern of reactivity observed with full-length hnRNP-H1 as antigen (data not shown). At a cut-off that corresponded to a specificity of 95%, antibodies to hnRNP-H1 Fragment 4 were found in 55.4% of patients with SS (*P* 0.0001, Mann–Whitney)

FIG. 1 Serum samples obtained from controls [CFS ($n = 106$], SSp ($n = 56$) and various autoimmune CTDs [DM ($n = 29$), MCTD ($n = 19$), PM ($n = 18$), SLE ($n = 71$), SSc ($n = 58$), RA ($n = 47$)] were tested for reactivity to a recombinant fragment of human hnRNP-H1 (amino acids 286–386) by western blot analysis. The figure shows the peak × area value as measured by densitometry for several patient groups. The figure shows box plots, from the first to the third quartile, with the addition of a notched section for the 95% CI around the median. The whiskers extend to the furthest observations within ±1.5 inter-quartile ranges (IQRs) of the first or third quartile. Observations outside 1.5 IQRs are marked as (+), and those outside 3.0 IQRs are marked as (*).



and in 31% of patients with a CTD other than SS (P 0.0001, Mann–Whitney). Reactivity to Fragment 3 was higher in patients with SS than in controls or patients with other CTDs ($P = 0.0002$, Kruskall–Wallis with Bonferroni correction), although the differences between the groups were less pronounced than the differences in reactivity between the groups observed for antibodies to Fragment 4. At a cut-off that corresponded to a specificity of 95%, antibodies to hnRNP-H1 Fragment 3 were found in 21.4% of patients with SS (P 0.0001, Mann–Whitney) and in 15.7% of patients with a CTD other than SS ($P = 0.048$, Mann–Whitney). In patients with SS, there was no association between RF, ANA, anti-SSA, anti-SSB or anti-U1RNP antibodies and antibodies to Fragment 4 or 3 (chi-squared analysis).

In conclusion, our data support the idea that RRM 3 is the major site of autoimmune recognition of autoantibodies to hnRNP-H1. The region from amino acid 198 to 285 also contains immunoreactive sites, though these are less immunoreactive than RRM 3.

Rheumatology key message

- Anti-hnRNP-H1 antibodies are directed to RNA recognition motif 3.

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First observation of the efficacy of IL-1ra to treat tophaceous gout of the lumbar spine

SIR, Tophaceous gout is a frequent cause of acute peripheral arthritis. Systematic review of the literature reveals 75 published cases that involve the spine. Our objective is to report a case of acute tophaceous gout of the lumbar spine mimicking spondylodiscitis with a dramatic symptomatic effect of interleukin 1 receptor antagonist (IL-1ra).

A 77-year-old woman was recently discovered with end-stage renal failure (serum creatinine was 360 μmol/l and glomerular filtration rate was 8 ml/min) due to a chronic obstruction (prolapse of the womb), recurrent urinary infections and chronic intake of NSAIDs for polyarthralgia. She had acute low back pain with arthritis of knees, elbows, hands and wrists, fever and biological inflammation (CRP was 360 mg/l, leucocyte count was 16 000/mm^3). She was referred to our Department of Rheumatology for suspicion of infectious spondylodiscitis. We found s.c. tophi on her fingers with concomitant hyperuricaemia (532 μmol/l). MRI of the lumbar spine showed a hypersignal T2 of the L4–L5 discus with high and heterogeneous enhancement of the vertebral plates associated with lateral effusion to the right psoas muscle. SFs and biopsy of the L4–L5 discus revealed numerous monosodium urate crystals and cultures remained sterile (Fig. 1). Thus, the diagnosis was chronic tophaceous gout with polyarthritis and lumbar spondylodiscitis, without evidence of infectious disease. Because of renal failure, NSAIDs were contraindicated and colchicin was avoided. Then as symptomatic treatment, she received IL1-ra (anakinra), 100 μg s.c. every other day. By the 10th day of treatment, the lumbar pain, number of swollen and