0.001). In both RA and AS, SAA correlates significantly with ESR ($r = 0.51$; $p = 0.0001$ in RA and $r = 0.61$; $p = 0.0001$ in AS), CRP ($r = 0.70$; $p = 0.0001$ in RA and $r = 0.66$; $p = 0.0001$ in AS) and IL-6 ($r = 0.58$ in RA; $p = 0.0001$, and $r = 0.48$ in AS; $p = 0.001$). In patients with RA, SAA but not CRP, ESR or IL-6, showed a positive, although weak correlation with the number of painful joints ($r = 0.31$; $p = 0.03$). Thompson articular index, but not the swollen joint count, correlates with the four parameters analysed; this correlation was better for CRP ($r = 0.51$; $p = 0.0001$) than for SAA ($r = 0.39$; $p = 0.007$) ESR ($r = 0.35$; $p = 0.02$) or IL-6 ($r = 0.37$; $p = 0.01$). **Conclusion** In both RA and AS, SAA correlates well with other laboratory markers (mainly with CRP) of inflammation. However SAA is not a better marker of clinical disease activity than CRP in RA.

THU0063 AUTOANTIBODIES TO HUMAN MYC FAR UPSTREAM ELEMENT-BINDING PROTEIN IN PATIENTS WITH RHEUMATOID ARTHRITIS

¹T Nojima, ¹A Suwa, ¹M Hirakata, ¹Y Ikeda, ²T Mimori. ¹Internal Medicine, Keio University School of Medicine, Tokyo, Japan; ²Clinical Immunology, Kyoto University, Kyoto, Japan

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Background We have shown that sera from patients with rheumatoid arthritis (RA) contain a variety of autoantibodies to unknown proteins such as 60-, 45-, and 75-kDa proteins, in preliminary immunoblot studies using HeLa cell extracts.¹ By molecular cloning of the target antigens, we have demonstrated that calpastatin (endogenous protein inhibitor of calpain, calcium-dependent cysteine proteinase) is one of such autoantigens and the presence of anti-calpastatin antibodies may be related to pathogenic mechanisms of RA.^{2,3}

Objectives We have attempted to identify autoantigens that were recognised by newly identified autoantibodies discovered in RA patient sera. We hereby describe that human myc far upstream element binding protein (FUSEbp) is a new target of autoantibodies in RA.

Methods Molecular cloning was performed using a lambda gt11 cDNA library constructed from HeLa cell mRNA and RA patient sera that contain autoantibodies to unknown 75-kDa proteins as probes. cDNA inserts of isolated clones were sequenced, and detailed homologies of nucleotide and amino acid sequences were analysed through the BLAST server at NCBI DNA database. Immunoblot analysis was examined using bacterial lysates prepared from lysogenic E.coli Y1089 carrying the recombinant lambda phages. The partial cDNA (position 192–975 of FUSEbp

mRNA, termed FUSEbp-A) was amplified by PCR and ligated into an expression vector (pMAL-C2X). The expressed FUSEbp-A-maltose binding protein fusion protein was analysed by immunoblotting for patient sera.

Results The cDNA (termed RA9–2) expressing the fusion protein recognised by RA probe sera was isolated, and a 1.2 kb insert of RA9–2 was found to show a 99% homology at amino acid level with FUSEbp. The deduced molecular mass of FUSEbp was identical with the size of the 75-kDa protein in HeLa cell extracts. However, this expressed bacterial lysates containing the lambda phage were reactive with only one of 72 RA (1%) and one of 36 SLE (3%) patient sera. In contrast, the FUSEbp-A fusion protein was recognised by 7 of 72 RA sera (10%) as well as the probe serum in immunoblot analysis.

Conclusion Human myc FUSEbp, regulatory factor of c-myc expression,⁴ may be a novel autoantigen recognised by sera from patients with RA. In RA, synovial fibroblasts exhibit elevated gene expression of proto-oncogenes such as c-myc. Although it remains unclear how autoantibodies to FUSEbp can be associated with etiologic and pathogenic mechanisms in RA, increased expression and release of FUSEbp may induce autoantibodies to FUSEbp in RA-specific synovial proliferation and cartilage destruction.

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THU0064 CLINICAL SIGNIFICATION OF ANTI-KU ANTIBODY

¹F Limbach, ²J Goetz, ¹RM Javier, ¹JL Kuntz, ¹J Sibilia. ¹Rheumatology; ²Immunopathology, CHU Hautepierre, Strasbourg, France

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Background The Ku autoantigen is a DNA binding factor consisting of 70 and 80 kDa protein which form a heterodimer involved in repair of ds DNA breaks. Since the original description of anti-Ku antibody in patients with polymyositis-scleroderma overlap syndrome, this antibody has been showed to be associated with several autoimmune diseases.

Objectives To describe clinical features of 8 patients with anti-KU antibody.

Methods

Results

Conclusion

Abstract THU0064 Table 1

	Patient 1	2	3	4	5	6	7	8
Age (years)	28	40	44	30	66	41	67	74
ENA (ELISA)	Ku/Ro	Ku	Ku/RNP/JO1	Ku	Ku/proteinase3	Ku	Ku	Ku
Clinical features	Digital vasculitis	Sicca syndrome, polyarthritis	Myalgia, pulmonary fibrosis, sclerodactylia, raynaud's phenom	Raynaud's phenom	Extra-capillar glomerulonephritis, polyarthralgia, rectal carcinoma	Polyarthritis	Bronchopneumonia, Pulmonary embolism	Hand's oedema, prostatic cancer
Diagnosis	Lupus	Sjögren syndrome	Myositis-scleroderma	Raynaud	Wegener granulomatosis	Rheumatoid arthritis	Chronic obstructive lung disease	Prostatic cancer RS3PE ?

- anti-Ku antibody is not specific of a connective tissue disease and can also be observed in patients without any autoimmune disease (n°7).
- In addition to previous described association, anti-Ku antibody can also be present in Wegener granulomatosis and idiopathic Raynaud's phenomenon.
- Two of our patients had an evaluative cancer (n°5 and 8) and one (n°7) presented a stomach neoplasm 3 years before the detection of anti-Ku antibody. This finding needs further investigations to assess if anti-Ku antibody can be associated with or predictive of a neoplasm.

THU0065 CLINICAL SIGNIFICANCE OF ANTI-ASPARAGINYL TRNA SYNTHETASE AUTOANTIBODIES

¹M Hirakata, ¹A Suwa, ¹T Nojima, ¹M Suzuki, ²S Nagai, ³T Mimori, ⁴IN Targoff. ¹Internal Medicine, Keio University School of Medicine, Tokyo; ²Respiratory Medicine; ³Clinical Immunology, Kyoto University, Kyoto, Japan; ⁴Medicine, University of Oklahoma, Oklahoma City, USA

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Background Autoantibodies to 5 of the aminoacyl tRNA synthetases (those for histidine, threonine, alanine, isoleucine and glycine) have been described, and each is associated with a similar syndrome of myositis (PM or DM) with interstitial lung disease (ILD), arthritis, and other features.¹ We have identified novel autoantibodies to asparaginyl tRNA synthetase (AsnRS),² but the clinical significance of this sixth anti-synthetase antibody has not been well-defined.

Objectives The aim of the present study was to identify patients with anti-AsnRS and elucidate the clinical and immunogenetic features of this sixth anti-synthetase autoantibodies.

Methods Sera from more than 2,500 patients with connective tissue disease including myositis and ILD in collaborating centres, and control sera were examined for anti-AsnRS antibodies by immunoprecipitation. Positive sera and controls were tested for the ability to inhibit AsnRS by preincubation of the enzyme source with the serum. The HLA-class II (DRB1, DQA1, DQB1, DPB1) alleles were identified from RFLP of PCR-amplified genomic DNA. The concentration of KL-6 (a new marker for ILD) in serum was determined by a specific ELISA.³

Results Anti-KS antibodies were identified in the sera of 8 patients (5 Japanese, 1 U. S., 1 German, 1 Korean), by immunoprecipitation of the same distinctive set of tRNAs and protein that differed from those precipitated by the other 5 anti-synthetases, and showed specific inhibition of AsnRS activity. Two of these patients had DM, but seven of 8 (88%) had ILD. Four (50%) had arthritis, and one had Raynaud phenomenon. This anti-synthetase was very rare among myositis patients, found in <0.2% of U. S. myositis patients, and 0% of Japanese myositis patients, but was found in 3% of Japanese ILD patients. Thus, most patients with anti-AsnRS antibodies had chronic ILD with or without features of connective tissue disease. Furthermore, four of 5 patients in whom KL-6 levels were measured, showed markedly elevated levels (mean level 919.8 U/ml; normal < 500 U/ml). Interestingly, all four Japanese patients tested had DR 2 (DRB1*1501/1502), compared to 33% of healthy controls.

Conclusion These results indicate that anti-AsnRS antibodies, like anti-PL-12 (alanyl tRNA synthetase) antibodies, have a stronger association with ILD than with myositis, and may be associated with the DR2 phenotype.

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THU0066 AUTOANTIGENIC EPITOPES TARGETED BY AUTOANTIBODIES TO RNA HELICASE A IN SYSTEMIC LUPUS ERYTHEMATOSUS

¹A Suwa, ¹M Hirakata, ¹T Nojima, ¹S Satoh, ²T Nakajima, ³T Mimori. ¹Internal Medicine, Keio University, Tokyo; ²Medical Research, St. Marianna Medical University, Kawasaki; ³Clinical Immunology, Kyoto University, Kyoto, Japan

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Background We have previously described new autoantibodies which recognise the 140 kDa polypeptide (p140) in a patient serum with SLE. Recently we have demonstrated that these antibodies are targeting RNA helicase A (RHA) which plays a pivotal role in nuclear events.

Objectives To investigate the target epitopes recognised by autoantibodies to RHA.

Methods Sera from 561 patients with systemic rheumatic disease (287 SLE, 71 SS, 67 PM/DM, 58 RA, 54 MCTD/overlap, 18 Sjogren syndrome and 6 vasculitis syndrome), and 80 normal humans were screened by immunoprecipitation assay using [35S]-Met-labelled HeLa cell extracts as antigen. In some experiments, [32P]-labelled HeLa cells were used to analyse nucleic acid components. To determine the antigenic epitopes on RHA, restriction fragments of cDNA encoding D1 (amino acids 1-250) containing double-stranded RNA binding domain, D2 (230-650) containing a DEAH box motif, D3 (630-1,020), and D4 (1,000-1,279) containing a glycine-rich region were subcloned into pGEX-2TK vector and expressed in *E. coli*. The resulting fusion proteins (PD1, PD2, PD3, and PD4) were detected with patient sera.

Results We have found 10 sera which immunoprecipitated a 140 kDa polypeptide from HeLa cell extracts in SLE patients with nephritis. When the precipitated polypeptides were used as antigen in immunoblotting, p140 was recognised by rabbit antisera against RHA. Thus, we have confirmed that all 10 sera contain autoantibodies to RHA. Moreover, it was noted that anti-RHA sera coprecipitated high molecular weight nucleic acids from [32P]-labelled HeLa cell extracts. When cell extracts were treated with RNase, these sera were unable to coprecipitate high molecular weight nucleic acids, indicating that nucleic acid components were RNAs. Under various salt conditions, anti-RHA antibodies were able to coprecipitate nucleic acid components at salt conditions lower than 0.35M NaCl. These results suggested that RHA is interacting with hn-RNA or mRNA. In immunoblotting using the fusion proteins (PD1, PD2, PD3, and PD4), all 10 anti-RHA positive sera strongly recognised PD4. Eight and 9 out of 10 sera recognised PD1 and PD3, respectively, but intensity of binding was variable among different sera. In contrast, none of sera reacted with PD2.

Conclusion Autoantibodies to RHA were identified exclusively in patients with lupus nephritis. The universal epitope was present on the carboxyl-terminal domain of RHA. The reactive patterns of antibodies against multiple antigenic determinants detected here were consistent with the hypothesis that relatively native RHA molecules directly elicit autoantibodies in selected patients.