THU0067 ANALYSIS OF AUTOANTIGENS RECOGNISED BY AUTOANTIBODIES THAT IMMUNOPRECIPITATE HIGH MOLECULAR WEIGHT NUCLEIC ACIDS

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Background A number of sera from patients with connective tissue diseases that immunoprecipitated high molecular weight (hwm) nucleic acid smear have been found in RNA-immunoprecipitation (IPP) assay. Most of these sera are supposed to contain autoantibodies for DNA- or mRNA- (or its precursor) related proteins. However, neither the characteristics of antigens nor the clinical significance have been well-defined.

Objectives To analyse the corresponding antigens for autoantibodies immunoprecipitating hwm-nucleic acid smear and to elucidate the clinical features associated with these autoantibodies.

Methods We examined 63 sera from patients with various kinds of connective tissue diseases that immunoprecipitated hwm nucleic acid smear by RNA-IPP. IPP using [35S] methionine-labelled HeLa cell extracts was performed for analysis of the protein components. Immunoprecipitated nucleic acid components were treated with DNase I or RNase A to identify either DNA or RNA.

Results DNA and a heterodimer of 70 kDa and 80 kDa proteins (p70/p80) that was identified as the Ku antigen, was immunoprecipitated by 16 patient sera (4:SLE, 7: myositis overlap syndrome, 1: interstitial pneumonia (IP), 4: undiagnosed). In addition, DNA-protein complex consisting of four polypeptides (11 kDa-21 kDa) that appeared to be histone core proteins, was immunoprecipitated by 18 sera (12:SLE including one drug-induced lupus, 5:MCTD/overlap syndrome, 1: autoimmune hepatitis). Eight patients sera (5:SLE, 2:RA, 1:IP) reacted with the 140 kDa protein along with hwm-RNA smear that was supposed to be RNA helicase A. It should be noted that the remaining 37 of 63 (59%) patients sera immunoprecipitated none of proteins with defined specificities, but various unidentified proteins.

Conclusion Autoantibodies to the Ku, histone, and RNA-helicase A have been identified in patients sera with SLE and other rheumatic diseases that immunoprecipitated hwm-nucleic acid smear. However, a high percentage of these sera were found to contain novel hwm nucleic acids-related antibodies. Further analysis of these autoantibodies may provide clues for understanding the pathogenic mechanism of connective tissue diseases.

Background Disc degeneration and physiopathology of disc herniation involves enzymatic processes mainly matrix metalloproteinases (MMPs), tissue inhibitor of metalloproteinase-1 (TIMP-1) and cytokines (IL-1b, TNF-a).

Objectives This study focuses on metalloproteinases secretion and expression by disc chondrocytes isolated from herniated and not herniated zone.

Methods Eleven discs after discectomy are enzymatically digested, chondrocytes from both zones, herniated and not herniated, are cultured in alginate beads for 10 days in classical conditions. Analysis of MMP-2,-3,-7,-9,-13 and TIMP-1 by ELISA, zymography, RT-PCR and nested PCR are conducted with an immunochemical study on tissue fragments.

Results - proteic level: (ELISA, zymography):

?presence of elevated levels of MMP-3, TIMP-1 in the non herniated part

?absence of MMP-13, MMP-9, pro MMP-7 in both parts

-Transcriptional level (RT, PCR, nested PCR):

?expression of MMP-2, -3, TIMP-1 in both zones, weak expression of MMP-9 by nested PCR without difference between the two zones.

?absence or weak expression of MMP-13, -7.

Conclusion There is an heterogeneous expression of MMP -2, -3, -9 and TIMP-1 by disc herniated and non herniated chondrocytes. The matrix metalloproteinases are probably involved early in the disc degeneration, the herniation could be the final result of matrix/cells interactions.

The secretion of TIMP-1 and MMP-3 in the herniated zone may be related discal fibrosis included into the disc cicatrization.

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AB0019 EFFECT OF EXTRACELLULAR SODIUM CONCENTRATION IN BIGLYCAN AND DECORIN SYNTHESIS BY HUMAN CHONDROCYTES

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Background An altered chondrocytes phenotypic expression of biglycan and decorin is an early event in osteoarthritis (OA). Changes in extracellular matrix sodium concentration ([Na⁺]) occurs in OA. Previous results suggested that chondrocytes are sensitive to changes in their extracellular [Na⁺] environment and respond with variations in the matrix synthesis.

Objectives Here we have studied the influence of extracellular [Na⁺] on the metabolism of small proteoglycans, biglycan and decorin, in a human chondrocyte-like cell line, C20/A4.

Methods Extracellular deposition of biglycan and decorin was analysed in C20A4 cells cultured: 1) immunohistochemically using specific antibodies against biglycan and decorin and

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AB0018 INTERVETEBRAL DISC HERNIATION: INVOLVEMENT OF METALLOPROTEINASES

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secondary IFTC conjugated antibodies; and 2) in SDS-PAGE electrophoresis and blotting using the corresponding antibodies.

Results Fluorescence immunohistochemically shows specific extracellular expression of biglycan and decorin in chondrocytes cultured in standard [Na⁺] (154 nm) and western blotting intensities of these small proteoglycans varied with [Na⁺].

Conclusion This study demonstrates that biglycan and decorin are expressed in the human chondrocyte cell line C20A4. We are now searching if [Na⁺] variations is able to produce qualitative or quantitative alterations in the matrix synthesis as it have been found in osteoarthritis.

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AB0020 COMPARISON OF SYNOVIAL MMP-1 AND TIMP-1 LEVELS IN PATIENTS WITH VARIOUS INFLAMMATORY ARTHRITIDES

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Background Some inflammatory arthritides like a rheumatoid arthritis cause erosion in bones and destruction in cartilage, but others such as Behçet's Disease (BD) and Familial Mediterranean Fever (FMF) do not.

Objectives The purpose of this study was to investigate synovial levels of matrix metalloproteinase 1 (MMP-1) known to break collagen, and tissue inhibitor of metalloproteinase (TIMP-1), its natural antagonist, in patients with various inflammatory disorders.

Methods One hundred and nine patients with different inflammatory arthritides were enrolled in the study (20 BD, 20 FMF, 24 spondyloarthropathy (SPA), 26 rheumatoid arthritis (RA) and 19 osteoarthritis (OA)). All patients had at least one-sided knee arthritis from which synovial fluid samples were obtained. Patients with BD, FMF and SPA constituted three study groups. Patients with RA and OA were used as control groups. Synovial MMP-1 and TIMP-1 levels were measured by two-step sandwich ELISA.

Results The mean levels of synovial MMP-1 and TIMP-1 of the study and control groups presented in Table 1.

Abstract AB0020 Table 1

	BD	Study Groups FMF	SPA	Control RA	Groups OA	p*
MMP-1	0.305 ±	0.237 ± 0.253	0.190 ±	0.244 ±	0.126 ±	0.019
mcg/dl	0.217		0.224	0.230	0.113	
TIMP-1	6.040 ±	5.784 ± 3.378	6.080 ±	5.660 ±	8.574 ±	0.017
mcg/dl	2.490		3.960	2.931	2.702	

MMP-1 and TIMP-1 levels of the study and control groups (*Kruskal-Wallis test).

Conclusion Although FMF and BD are generally characterised by nonerosive arthritis, our results showed that synovial MMP-1 levels of patients with BD and FMF were not different from the patients with RA, which is a well-known erosive arthritis. We suggested two possible explanations for these unexpected data. First, BD and FMF generally cause short term arthritic attacks, remitting without permanent damage. Second, high synovial MMP-1 levels in BD and FMF may be resulted from extra

articular origin, because of their systemic nature. The comparison of synovial MMP-1 levels between RA and SPA was statistically significant. This may help to explain why SPA causes less prominent destruction in cartilages and erosion in bones than RA. Except OA, the difference between the groups with respect to the synovial TIMP-1 levels was statistically insignificant.

AB0021 TOPIRAMATE AS SUSPICIOUS CAUSE OF ARTHROPATHY- ANOTHER CASE REPORT

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Background One year ago we described the case of a 23 year old female suffering from idiopathic generalised epilepsy treated with valproate and topiramate. The patient developed a poliartthritis that improved after topiramate was discontinued.¹

Objectives

Methods We present a 58 year old male suffering since 1996 from epileptic seizures and left hemiplegia secondary to subarachnoid haemorrhage; he had a history of hypertension, diabetes and cigarette smoke.

Results Since September 1999 the patient was treated with phenobarbital at the dose of 100 mg per day, since January 2000 topiramate was added at the dose of 100 mg per day. He was admitted in our department in July 2000 because of worsening of the motor performance: due to myalgias of the limbs, arthralgias to the left knee and hand, and a frozen left shoulder (it was impossible to obtain any passive movement of that joint). X-ray of the left shoulder showed Sudeck's atrophy; rheumatological blood tests were negative. We stopped topiramate and substituted it with gabapentin at the dose of 100 mg t.i.d.. 3 months after suspension of topiramate, continuing the treatment with gabapentin and phenobarbital, the patient showed a marked improvement in the range of motion of the left shoulder (passive abduction of 45°; because of hemiplegia, active movements were impossible); moreover arthralgias and myalgias at the limbs disappeared.

Conclusion Topiramate, a new anti-epileptic drug, seems to have a potential arthropathic role which should be better studied.

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THU0052 FIBROBLAST-LIKE SYNOVIOCYTES FROM RHEUMATOID ARTHRITIS PATIENTS ARE MORE INVASIVE THAN FROM OSTEOARTHRITIS IN AN IN VITRO SYSTEM: CORRELATION WITH GROWTH CHARACTERISTICS AND EXPRESSION OF MMP-1, MMP-3 AND MMP-10

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Background

Objectives Fibroblast-like synoviocytes (FLS) are involved in joint destruction. In this study the invasiveness in a transwell