Background Genetic polymorphisms, such as the polymorphisms of vitamin D receptor (VDR) and oestrogen receptor (ER) genes play an important role in bone metabolism and the pathogenesis of osteoporosis. Little is known about the role of VDR and ER gene polymorphisms in rheumatoid arthritis (RA), although hormonal factors are highly involved in the evolution of autoimmune disorders.

Objectives We assessed VDR BsmI, ER XbaI and ER PvuII polymorphisms in 50 RA patients as well as 50 healthy subjects.

Methods Gene polymorphism was assessed in peripheral blood mononuclear cells taken from RA patients and healthy controls. After the PCR amplification of DNA isolated from these cells using specific primer pairs, DNA was digested with BsmI, PvuII and XbaI restriction enzymes. B versus b, X versus x and P versus p alleles, as well as homozygote and heterozygote genotypes were determined by agarose gel electrophoresis. Genotypes were then correlated with the bone density (assessed by DEXA), serum osteocalcin and urinary pyridinolin crosslink concentrations. Disease activity of RA was assessed by ESR and CRP.

Results There is difference between VDR and ER polymorphisms in RA patients versus controls. A significantly higher percentage of RA patients carry the VDR Bb heterozygote genotype. XbaI and PvuII ER genotypes are also different in RA patients versus controls. There is a correlation between the VDR and ER genotypes, as well as bone density, biochemical markers and laboratory markers of RA activity.

Conclusion It is likely, that VDR and ER polymorphisms are involved in bone metabolism as well as inflammatory events underlying RA.

THU0011 BSM-1 POLYMORPHISM AND BONE MINERAL DENSITY IN POLYMYALGIA RHEUMATICA

¹A Daroszewska, ¹W Taylor, ¹JL Usher, ²RC Bucknall, ³RJ Moots, ³RN Thompson, ²EJ Tunn, ¹WD Fraser. ¹Department of Clinical Chemistry, The University of Liverpool; ²Department of Rheumatology, Royal Liverpool University Hospital; ³Department of Rheumatology, University Hospital Aintree, Liverpool, UK

10.1136/annrheumdis-2001.114

Background VDR gene polymorphism has been associated with variations in bone mineral density (BMD) in normal populations, most studies linking the presence of the Bsm-1 restriction site (b) with higher BMD as compared to absence of Bsm-1 restriction site (B). We found that in steroid induced osteoporosis in PMR patients there is an overrepresentation of bb and that in normal controls, higher BMD is found in Bb heterozygotes (FA Gidden: data submitted).

Objectives To investigate the VDR gene polymorphism frequency and association with BMD in newly diagnosed patients with PMR, prior to treatment with corticosteroids.

Methods Thirty patients with active PMR (fulfilling Bird's criteria), consecutively referred by GP's to the PMR clinic, underwent lumbar spine and hip BMD scanning on a Hologic dual X-ray absorptiometer (DXA). 5 ml EDTA blood was obtained from each patient and used for DNA extraction. Polymerase chain reaction (PCR) and agarose gel electrophoresis were used for Bsm-1 site typing.

Results The VDR genotype distribution was: BB in 6 patients (20%), Bb in 10 patients (33.3%) and bb in 14 patients (46.7%). Mean BMD of lumbar spine (L1-L4) expressed as Z-score was in BB group 0.32 (95% CI: -0.47–1.11), in Bb group 1.37 (95% CI: 0.31–2.41) and in bb group 0.46 (95% CI: -0.25–1.18). Mean BMD of femoral neck (Z-score) was in BB group -0.05

(95% CI: -1.5–1.39), in Bb group 0.49 (95% CI: -0.24–1.23) and in bb group 0.13 (95% CI: -0.39–0.65). Mean age of patients was 73.6, SD 7.6. There was no statistically significant difference between groups with regard to age, disease duration and disease activity (measured by ESR, CRP, Pain Visual Analogue Score, Global Disease Activity Score and Health Assessment Questionnaire).

Conclusion There is an overrepresentation of bb in PMR patients (as compared to UK VDR frequencies: BB 20.5%, Bb 49.7%, bb 29.8%)¹ which may reflect VDR polymorphism role in susceptibility to inflammatory conditions e.g. PMR. Heterozygotes Bb have highest BMD. Bb genotype may be bone protective for steroid induced osteoporosis.

REFERENCE

¹ Houston LA, et al. *Bone* 1996;**18**(3):249–52

THU0012 CLONAL ANALYSIS OF VH AND VL MRNA IN SINGLE B AND PLASMA CELLS FROM RHEUMATOID ARTHRITIS SYNOVIUM AND PERIPHERAL BLOOD

^{1,2} Ruzickova, ¹O Krystufkova, ¹Z Cimburek, ¹J Niederlova, ³J Sinkora, ⁴O Horvath, ²T Doerner, ¹J Vencovsky. ¹Institute of Rheumatology and Laboratory of Gene Expression; ²Department of Rheumatology/Immunology, Medical Faculty, Charite, Humboldt University, Berlin, Germany; ³Division of Immunology and Gnotobiology; ⁴Department of Immunology, Institute of Microbiology CAS, Prague, Czech Republic

10.1136/annrheumdis-2001.115

Background Rheumatoid arthritis (RA) is characterised by a complex infiltration of many cell types including T and B lymphocytes, plasma cells, follicular dendritic cells, macrophages and other cells into synovial tissue of inflamed joints. Presence of B and plasma cells results in a local synthesis of multiple pathologic immunoglobulins contributing to the chronic joint inflammatory processes.

Objectives To analyse mRNA from individual B and plasma cells isolated from synovium and peripheral blood of patients with RA, in order to investigate a clonality, VH and VL pairing and a molecular structure of produced antibodies.

Methods RA synovial tissue after synovectomy was enzymatically digested. Single synovial B/plasma cells were sorted using immunofluorescent staining with anti-CD19, anti-IgM and anti-CD38. RA peripheral B cells were isolated in the same way without enzyme treatment. cDNA library from each single B and plasma cell was generated. Nested polymerase chain reaction was performed to analyse VH and VL gene usage. VH, DH and JH or VL and JL gene segments were assigned and somatic mutations determined by comparison to germline sequences on the V BASE/Genbank database. As a control peripheral B-lymphocytes from healthy donor were screened.

Results Analysis of RA synovial mRNA revealed prevalence of C gamma rearranged transcripts that contained rearranged VH1, VH2, VH3, VH4, VH5 and VH6 genes. Utilisation of VH segments was similar between RA patients and normal subjects, but the accumulation of somatic mutations was elevated in RA synovial and peripheral B cells. Preferential utilisation of a limited number of VH and DH gene segments was found. There was an increased frequency of kappa light chains containing unusually long CDR3 when compared to normal peripheral B cells. mRNAs resulting from transcription of non-productive rearrangements were also detected as well as transcripts corresponding to isotype-switching.

Conclusion Our findings are consistent with hypothesis that B cell response in RA synovium is probably antigen driven and oligoclonal.

The project was supported by grant VS96129 from Czech Ministry of Education, Youth and Sport in the Czech Republic.

SLE – Clinical aspects

AB0071 LYMPHOCYTOTOXIC ANTIBODY (LCA) AMONG IRAQI SLE PATIENTS AND THEIR RELATIVES

¹MM Hasan, ²KM Mousawy, ²S Salman, A Al-Wahab, ²AR Al-Shaikly. ¹Faculty of Pharmacy

10.1136/annrheumdis-2001.116

²Faculty of Medicine, Baghdad University, Baghdad, Iraq

Background

Objectives To study the Lymphocytotoxic antibodies (LCA) in SLE patients and their first degree relatives compared to matching controls.

Methods Complement dependent microcytotoxicity test was used to assess the presence of LCA, their temperature dependence in addition to HLA specificity among 40 SLE patients, 184 of their first degree relatives in comparison to 100 subjects of a blood bank donor as a healthy control.

Results Lymphocytotoxic activity was manifested in 70% of patients, 33.36% of relatives compared to 80% of control group. While cold dependent antibodies were higher among patients group (92.8%) compared to 35% of their relatives. The presence of these antibodies in a control group was less temperature dependent, since 62.5% of them reacted both at 22°C and 15°C and only 12.5% of them exhibited a sole reactivity of 15°C.

No association between LCA and and of HLA antigen of both classes was detected, however, patients had a tendency to react with a large number of lymphocyte samples than did positive sera from other group. Generally, females had a higher percentage of LCA (42.15%). Yet, the highest percentage was reported among sisters, followed by mothers, while brothers exhibited the least frequency (15.15%). In contrast to females, male subjects reported the highest frequency of these antibodies among age group of > 50 years.

Conclusion Patients expressed a high positivity rate of cold LCA. Moreover, female first degree relatives showed higher positivity rates than male relatives or controls.

AB0072 LYMPHOCYTOTOXIC ANTIBODY (LCA) AMONG IRAQI SLE PATIENTS AND THEIR RELATIVES

¹AA Al-Shaikly, ²MM Hasan, ¹KM Mousawy, ¹S Salman. ¹Faculty of Medicine; ²Faculty of Pharmacy, Baghdad University, Baghdad, Iraq

10.1136/annrheumdis-2001.117

Background

Objectives To study the lymphocytotoxic antibodies (LCA) in SLE patients and their first degree relatives compared to matching controls.

Methods Complement dependent microcytotoxicity test was used to assess the presence of LCA, their temperature dependence in addition to HLA specificity among 40 SLE patients, 184 of

their first degree relatives in comparison to 100 subjects of a blood bank donor as a healthy control.

Results Lymphocytotoxic activity was manifested in 70% of patients, 33.36% of relatives compared to 80% of control group. While cold dependent antibodies were higher among patients group (92.8%) compared to 35% of their relatives. The presence of these antibodies in a control group was less temperature dependent, since 62.5% of them reacted both at 22°C and 15°C and only 12.5% of them exhibited a sole reactivity of 15°C.

No association between LCA and any of HLA antigen of both classes was detected, however, patients had a tendency to react with a large number of lymphocyte samples than did positive sera from other group. Generally, females, had a higher percentage of Lca (42.15%), yet, the highest percentage was reported among sisters, followed by mothers, while brothers exhibited the least frequency (15.15%). In contrast to females, male subjects reported the highest frequency of these antibodies among age group of > 50 years.

Conclusion Patients expressed a high positivity rate of cold LCA. Moreover, female first degree relatives showed higher positivity rates than male relatives or controls.

AB0073 A CASE OF FULMINANT LUPUS IN A YOUNG MAN WITH HEREDITARY HAEMOCHROMATOSIS

¹CF Matthews, ²P Gardiner. ¹Rheumatology, Musgrave Park Hospital, Belfast; ²Medicine, Altnagelvin Hospital, Londonderry, UK

10.1136/annrheumdis-2001.118

Background Mild derangements of liver function tests in systemic lupus erythematosus (SLE) are not uncommon, reflecting either disease activity or treatment. Rare causes of hepatic dysfunction include arteritis and lupoid hepatitis.¹ We report on a patient with SLE and coexisting haemochromatosis.

Objectives

Methods CASE REPORT: A 26 year old man presented with acute onset of jaundice and skin rash. He had a history of intermittent rash and arthralgia with serology positive for ribonuclear protein antibodies characteristic of mixed connective tissue disease. On examination he was icteric with a pyrexia. There was a maculopapular rash typical of subacute cutaneous lupus over his anterior chest wall.

Results Investigations showed a normocytic, normochromic anaemia, a thrombocytopaenia and a lymphopaenia. His prothrombin time and partial thromboplastin time were prolonged. Liver function tests showed an elevated total bilirubin and raised aspartate transferase and alanine transferase. Autoimmune serology showed positive antiRNP/Sm. Complement levels were low. Anticardiolipin antibodies were negative. Viral titres were negative. Serum ferritin level was elevated. Blood cultures grew *Staphylococcus aureus*. He was treated with high dose corticosteroids. He had intravenous antibiotics. The coagulopathy was treated with vitamin K and fresh frozen plasma. The thrombocytopaenia was treated with immunoglobulins. In spite of further management, unfortunately he died. A postmortem showed acute renal tubular necrosis and crescentic glomerulonephritis. Liver histology showed periportal deposition of iron predominantly in zone three. This, together with the presence of iron deposition in the exocrine pancreas gland confirmed the diagnosis of hereditary haemochromatosis.