THU0002 IL-10 AND IL-6 POLYMORPHISMS ARE NOT RELATED TO EROSIVE DISEASE IN EARLY RHEUMATOID ARTHRITIS

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Background

Objectives To assess the prevalence of IL-10 and IL-6 polymorphisms in patients with early rheumatoid arthritis (RA) in our population and investigate whether there is an association with radiographic severity.

Methods 68 patients with early RA (49F/19M, mean age 51 ± 15 y, mean disease duration 15 ± 13 mo, RF+ 72%, mean number of swollen joints at entry 8.23 ± 7.33). Patients were genotyped for HLA-DRB1 and IL-10 and IL-6 alleles and results compared with the control population (n = 110). Hands and feet radiographs were taken at entry and then yearly during follow-up. The Larsen method was used to assess radiographic damage.

Results There were not differences in the distribution of IL-6 alleles in the RA patients compared with controls. A difference was found in the distribution of IL-10 polymorphism at position ?592 between patients with RA and the control population: 592CC, 592CA and 592AA were found in a 40%, 54% and 6% of patients with RA versus a 55%, 33% and 12% respectively among controls (p = 0.018). No other differences were found in the frequency of the IL-10 alleles between RA and controls. No associations related to IL-6 or IL-10 polymorphisms were found in relation to radiographic damage.

Conclusion The IL-10 polymorphism at position ?592 showed a greater proportion of the CA allele in AR compared with the control population, although the clinical implications if any of this difference remains to be determined. No IL-6 nor any other IL-10 polymorphisms either were found to be associated to RA. No IL-10 nor IL-6 polymorphisms were found to be related to erosive disease in early rheumatoid arthritis.

THU0003 ALTERED GENE EXPRESSION IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS AFTER FEW CULTURE PASSAGES

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Background

Objectives Inflammation, synovial hyperplasia and articular destruction are typical features of rheumatoid arthritis (RA) pathophysiology modulated by activated synovial fibroblasts. To evaluate distinct metabolic pathways and gene expression, for the majority of experimental settings cultured RA synovial fibroblasts derived from synovial tissue samples are used. To analyse, whether RA synovial fibroblasts can be cultured for an extended period of time without changes in their gene expression pattern, a combination of RNA arbitrarily primed PCR (RAP-PCR) and cDNA array was used.

Methods RA synovial fibroblasts of 2 patients with RA were isolated and cultured under standard conditions for up to 9 passages. RNA was extracted from these cells after each of the passages 2–9. RAP-PCR was performed and the PCR reactions

were hybridised to cDNA expression array membranes (Atlas™, Clontech). For gene expression analysis, cDNA expression array membranes with 190 tumour suppressor genes and oncogenes and 9 control genes were used. The gene expression pattern of RA samples after each passage was compared to the pattern of the previous and following passages.

Results RA synovial fibroblasts showed a stable cDNA expression pattern for passages 2 to 4. After passages 5 to 6, expression pattern started to change (approximately 5% of the expressed genes). After passages 7 to 8, expression pattern of more than 5% of the 190 analysed tumour suppressor genes and oncogenes were altered. Of these, oncogene (e.g. bcl-1) and tumour suppressor-associated gene (e.g. EB-1 and CDK tyrosine kinase) expression had changed. Of interest, cell proliferation and growth decreased after passages 6 to 7 but distinct oncogenes and tumour-suppressor genes (e.g. jun-B and bcl-1) were still found to be upregulated.

Conclusion The results of this study reveal that gene expression of cultured RA synovial fibroblasts starts to alter after few passages indicating that future experimental settings need to address this potential problem for interpretation of gene expression. In addition, the data support the hypothesis that lack of downregulation of oncogenes and tumour suppressor genes might be responsible for the long-term aggressive growth of RA synovial fibroblasts observed in RA joints.

THU0004 ASSOCIATION BETWEEN HLA-DRB1 POLYMORPHISM AND MARKERS OF SEVERITY OF RHEUMATOID ARTHRITIS

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Background It is now well established that genetic variations in HLA-DRB1 region may influence susceptibility to rheumatoid arthritis (RA). However genetic component of the disease severity has not been fully elucidated.

Objectives The goal of the study was to assess the association of the HLA-DRB1 gene with different markers of severity of the disease.

Methods Seventy six patients with RA and one hundred fifty two healthy controls were typed for HLA-DRB1 gene by molecular method (PCR-SSO). Clinical, radiographic and immunological assessment of patients was carried out. In order to compare disease progression in patients with different disease duration we introduced joint destruction index (JDI) defined as the number of eroded joints (II-IV° Steinbrocker's stage) per years of the disease duration.

Results DRB1*04 alleles were present in 47% of RA patients and in 25% healthy controls (OR = 2,7; p_{cor} = 0,01). The frequency of DRB1*04 alleles was higher in patients with: 1) rheumatoid factor (RF) positive RA vs RF negative RA (OR = 14,9; p_{cor} = 0,001); 2) primarily chronic course of disease vs acute course of disease (OR = 4,7; p_{cor} = 0,03) and familial incidence of RA comparing to patients without familial history of RA (OR = 7,8; p_{cor} = 0,02). DRB1*04 alleles were significantly more frequent in patients with more intensive joint destruction reflected by JDI equal or higher than 1 (OR = 12,2; p_{cor} = 0,001). We did not observed differences in frequencies of DRB1*01 alleles neither between patients and controls nor between subgroups of RA patients.

Conclusion Our study confirmed association of DRB1*04 alleles not only with susceptibility to RA but with clinical features of the disease as well.

THU0005 INCREASED FREQUENCY OF HLA DR 11 AND DECREASED FREQUENCY OF HLA DR 1 IN CAUCASIAN EUROPEANS WITH ABDOMINAL AORTIC ANEURYSMS

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Background A genetic predisposition has been reported to play a key role in the pathogenesis of abdominal aortic aneurysms (AAA) in Japanese and a subgroup of North American patients with inflammatory AAA.

Objectives The aim of this study was to evaluate a possible association of HLA DR phenotypes with AAA in a European population sample.

Methods Consecutive patients with AAA larger than 3 cm presenting at our outpatient clinic were analysed for HLA class II genotypes using the PCR-SSP technique at low resolution level. DR phenotypes were deduced from these results. Patients with any history of an autoimmune disease were excluded from the study. The frequencies of HLA DR phenotypes were compared to those of a cohort of consecutive and ethnically matched organ donors using the appropriate statistical methods. Classification trees have been applied to increase the predictive value and to consider more than one variable.

Results HLA DR genotyping was performed in 54 European AAA patients (44 males) and 296 controls (203 males). At the HLA DRB1* locus, 19/54 AAA patients (35,2%) and 63/296 controls (21,3%) were found to be 1*11 positive ($p = 0,035$; OR = 2,01, 95% c.i.: 1,08–3,75). Subanalysis of male patients revealed 17/44 (38,6%) AAA patients and 39/203 (19,2%) in controls to be 1*11 positive ($p = 0,009$; OR = 2,65, 95% c.i.: 1,31–5,33). Regarding HLA DRB1*01, 4/54 AAA patients (7,4%) and 64/296 controls (21,6%) turned out to be 1*01 carrier ($p = 0,014$; OR = 0,29, 95% c.i.: 0,10–0,83). Subanalysis of male patients revealed 2/44 (4,5%) and 48/203 (23,6%) DRB1*01 carrier in AAA patients and controls, respectively ($p = 0,003$; OR = 0,15, 95% c.i.: 0,04–0,66). No associations for DRB1*11 and 1*01 were observed in female patients.

Conclusion These data suggest a positive association between AAA and DR 11 and a possible protective effect of DR 1 in male Caucasian Europeans suffering from AAA. This finding supports the hypothesis that MHC class II genes play a role in the pathogenesis of AAA.

THU0006 DQB1 POLYMORPHISM IN RHEUMATOID ARTHRITIS PATIENTS WITH SHARED EPITOPE-NEGATIVE DRB1 ALLELES

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Background The association of rheumatoid arthritis (RA) with HLA-DRB1 alleles sharing sequences in the third hypervariable region has been reported in several investigations, but genetic

analyses suggest the possible involvement of other HLA class II genes in conferring susceptibility.

Objectives We have investigated the distribution of HLA-DQB1-DRB1 polymorphism in 87 RA patients from southern Croatia and 141 healthy controls. RA patients were predominantly females (83 females vs 5 males).

Methods All patients and controls were tested for HLA class II alleles (DRB1, DQA1, and DQB1) by PCR-SSP method.

Results Distribution of predisposing DRB1 alleles according to the shared epitope (SE) model showed that 11 patients were homozygous for the predisposing alleles. Additional 48 patients carried one of the SE predisposing alleles (*0101, *1001, *0401, *0404, *0405, *0408). Distribution of DQB1 alleles among patients with SE-positive DRB1 alleles revealed that all DRB1*0101 and *1001 patients were DQB1*0501 positive. Among twenty patients with SE-positive DRB1*04 alleles, three different DQB1 alleles were observed (DQB1*0301, *0302 and *0201). Remaining 26 patients with SE-negative DRB1 alleles were additionally analysed for the presence of DQB1*0301, *0302 and *0201 alleles. All eight patients with SE negative DRB1*04 alleles (DRB1*0402, *0403, *0407) were DQB1*0302 positive. Among the remaining non DRB1*04 patients, 16 were either DQB1*0301 or *0201 and only two patients did not carry neither DQB1*0301 nor *0201.

Conclusion These data suggest that in patients with SE negative DRB1 alleles, DQB1*0301, *0302 and *0201 alleles should be considered as a genes contributing to the RA predisposition. DQB1*0301, *0302 and *0201 alleles should be considered as a genes contributing to the RA predisposition.

THU0007 ASSOCIATION OF HUMAN MHC CLASS I AND CLASS II GENES WITH SUSCEPTIBILITY TO SYSTEMIC LUPUS ERYTHEMATOSUS IN MALAYS AND CHINESE

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Background Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder. Its prevalence is higher in Chinese and Black populations compared to Caucasians. The prevalence of SLE in Malaysia is estimated to be 43/100,000 population.¹

Objectives The objective of this study was to investigate the association between SLE with the human MHC Class I and Class II genes.

Methods A modified PCR-SSP Methodology, known as Phototyping^{2,3} was carried out to determine the HLA-A, -B, -DR and -DQ genes of the subjects. 50 Malays and 50 Chinese patients with SLE fulfilling the ACR criteria were recruited along with 50 Malays and Chinese age- and race-matched controls.

Results Statistical analysis showed that both the Malay and Chinese have similar results despite minor differences. HLA-A11 is associated with SLE in both races, whereas HLA-DR12 has been found to be statistically significant as protective antigen specificity. Compared to Malays, Chinese SLE patients have a higher frequency of DR2 compared to the controls. DRB1 was only seen in Malay SLE patients (OR 8.69 compared to controls). DRB1*02 and DQB1*05 was only seen in Chinese SLE patients (OR 2.41 compared to controls). Alleles negatively associated with SLE, possibly conferring a protective effect are as follows: In both Malay and Chinese SLE patients, DRB1*12,